1. TITLE PAGE

SmartPractice

Final Report for Protocol SP 12 1PL 201: Clinical Evaluation of Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral®) Dose Response Study

Name of Investigational Product:	T.R.U.E. TEST
Indication Studied:	Diagnosis of allergic contact dermatitis
Protocol Number:	SP 12 1PL 201
Sponsor:	SmartPractice 3400 E. McDowell Rd. Phoenix, AZ 85008
Sponsor Contact:	Curt Hamann, M.D. Medical Director Telephone: (800) 365-6868
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Version of Report:	1.0
Study Dates:	18 February 2013 (first subject enrolled) to 17 June 2013 (last subject exited)

This study was performed in compliance with the ethical principles of the Declaration of Helsinki and Good Clinical Practices (GCP) including the archiving of essential documents.

APPROVAL STATEMENT AND SIGNATURES

Final Report for Protocol SP 12 1PL 201: Clinical Evaluation of Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral®) Dose Response Study

I have read this report and confirm that, to the best of my knowledge, it accurately describes the conduct and results of the study.

SIGNATURE: _____ DATE: 2-27-14

Curt Hamann, M.D.
Medical Director,
SmartPractice

SIGNATURE: _____ DATE: 3-3-14

James P. Bowman, M.S.
James Bowman & Associates, LCC

2. SYNOPSIS

Name of Sponsor/Company: SmartPractice	**Name of Active Ingredient:** Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral®)	**Name of Finished Product:** T.R.U.E. TEST investigational Lyral® allergens
Title of Study: Clinical Evaluation of Hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyral®) Dose Response Study		
Investigators: Evy Paulsen, M.D., Ph.D. (investigator) and Charlotte G. Mortz, M.D., Ph.D. (sub-investigator)		
Study Centers: This study was conducted at one investigational site located in Denmark (E Paulsen).		
Publication (reference): Not applicable		
Studied Period (years): 18 February 2013 (first subject enrolled) to 17 June 2013 (last subject exited)		
Objectives: The primary objective of the study was to determine the lowest concentration of ascending doses (0.40 mg/cm^2, 0.20 mg/cm^2, and 0.10 mg/cm^2) of hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyral®) in a Thin-layer Rapid Use Epicutaneous Test (T.R.U.E. TEST) panel to elicit +1 or +2 positive reactions in 70-90% of 20 adult "sensitive" subjects with a clinical history of contact dermatitis and a positive patch test (current or previous) to either Lyral® or Fragrance mix 2. The secondary objective of the study was to evaluate the safety of Lyral® in 20 adult "sensitive" subjects with a clinical history of contact dermatitis and a positive patch test (current or previous) to either Lyral® or Fragrance mix 2.		

Name of Sponsor/Company:	**Name of Active Ingredient:**	**Name of Finished Product:**
SmartPractice	Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral®)	T.R.U.E. TEST investigational Lyral® allergens

Methodology:

This was a prospective, single-center, double-blind, randomized clinical trial designed to compare the diagnostic performance (primary objective) and safety (secondary objective) of ascending concentrations of Lyral® (0.40 mg/cm^2, 0.20 mg/cm^2, and 0.10 mg/cm^2) on a T.R.U.E. TEST panel in 20 adult subjects with clinical histories of contact dermatitis and a positive patch test (current or previous) to Lyral® or Fragrance mix 2.

On Day 0 (Visit 1) all eligible subjects had two panels placed on their back. One panel contained three chambers filled with one of three concentrations (0.40 mg/cm^2, 0.20 mg/cm^2, and 0.10 mg/cm^2) of Lyral® in β-cyclodextrin and polyvinylpyrrolidone (PVP) and a fourth chamber that served as a negative control (PVP only). The sequence of the doses and negative control on the panel were rotated randomly on the panel into three different configurations. Although the investigators were aware of the doses and allergen on the panel, they were unaware of the specific location of the doses or negative control on the panels. The second panel contained the reference allergen (20 mg of Lyral® 5%, in petrolatum). Because the reference allergen was on a separate panel, its location could not be blinded. The panels were worn for 48 hours.

After 48 hours (Visit 2), the adhesion of the test panels was assessed and then they were removed. The skin was allowed to rest for 20 minutes after which all test site skin reactions were evaluated along with any tape irritation. Subjects' reports of itching and/or burning at the test sites were recorded.

Additional evaluations of test site skin reactions were conducted at 4 days -1 day (Visit 3), 7 days + 1 day (Visit 4), and 21 days ± 2 days (Visit 5) after the initial placement of the patch panels. At each of these evaluations, ndd adverse events (AEs) were documented. At Visits 4 and 5, late and/or persistent skin reactions were recorded. All subjects exited the study at the completion of Visit 5, which could have been conducted over the telephone.

Number of Subjects (planned and analyzed):

Planned = 20/Enrolled = 22 Analyzed = 22 for safety endpoints and to support efficacy endpoints (intent-to-treat (ITT) population and 20 subjects who completed the study with no major protocol violations (per protocol (PP) population) for efficacy endpoints.

Diagnosis and Main Criteria for Inclusion:

The study population was planned to include 20 adult subjects, 18 years of age and older, with a clinical history of contact dermatitis and a positive patch test (current or previous) to Lyral® or Fragrance mix 2. Subjects must have been otherwise healthy and able or willing to fulfill the entry criteria. In general, the enrollment sought to include at least 50% (but not more than 85%) female subjects.

Test Product, Dose and Mode of Administration, Batch Number:

Test Article: T.R.U.E. TEST panel with allergen and negative control patches that included:

- Lyral® in PVP, 0.10 mg/cm^2
- Lyral® in PVP, 0.20 mg/cm^2
- Lyral® in PVP, 0.40 mg/cm^2
- PVP (negative control)

Administration: Topical patch application

Batch Number: 13001

Duration of Treatment:

Patches removed two days after application

Name of Sponsor/Company:	**Name of Active Ingredient:**	**Name of Finished Product:**
SmartPractice	Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral®)	T.R.U.E. TEST investigational Lyral® allergens

Reference Therapy, Dose and Mode of Administration, Batch Number:

Reference Article: Lyral® (Hydroxyisohexyl 3-cyclohexene-1-carboxaldehyde), 5% in petrolatum, 20 mg

Administration: Topical patch application within patch test chambers

Batch Number: 13001

Criteria for Evaluation:

Primary Endpoints:

The diagnostic performance of Lyral® T.R.U.E. TEST allergens included the following:

- Determination of the lowest allergen concentration that elicited a positive skin reaction (+1 or +2) in 70% to 90% of the sensitive subjects (i.e., subjects who were sensitive to Lyral® at entry). A positive reaction was determined by the investigator as a positive test site skin reaction observed at Visit 3, Visit 4, or Visit 5.
- Frequency of positive, negative, doubtful, and irritant reactions for each concentration of Lyral®, and for the negative control, and reference allergen.
- Concordance and discordance between each concentration of the Lyral® T.R.U.E. TEST allergen and the reference allergen in petrolatum and among the three dose of the Lyral® T.R.U.E. TEST allergen.

Secondary Endpoints:

The safety performance of Lyral® T.R.U.E. TEST allergens included the following:

- Tape irritation at patch test removal.
- Panel adhesion.
- Itching and burning at patch test removal.
- Late reactions.
  A late reaction was defined as a positive test site skin reaction that initially occurred 7-10 days after application of the patch panel and chamber.
- Persistent reactions.
  A persistent reaction was defined as a positive test site skin reaction that appeared at Day 2 and/or Day 3 and /or Day 4 and persisted through Day 7 or to Day 21.
- Hyperpigmentation, hypopigmentation, pruritus, or other reactions.
- Other AEs.
- Severe AEs.

Statistical Methods:

All statistical processing was performed using SAS software unless otherwise stated. All computations and tabulations were conducted using all enrolled subjects. In this study, “sensitive subjects”were subjects who had positive baseline (or historical) patch test reactions to Lyral® in petrolatum or to Fragrance mix 2). No imputations for missing data were performed.

Name of Sponsor/Company:	**Name of Active Ingredient:**	**Name of Finished Product:**
SmartPractice	Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral®)	T.R.U.E. TEST investigational Lyral® allergens

Demographics and Baseline Characteristics

Demographic (age, gender) and baseline characteristics (previous positive patch test results, type of dermatitis, presence of current dermatitis symptoms, and dermatitis symptom areas) of all enrolled subjects were summarized using descriptive statistics. Quantitative measures were summarized using the number of subjects (N), along with the mean, standard deviation (STD), median, minimum, and maximum. Qualitative measures were summarized using frequency counts and percentages. All medical histories and concomitant medication usages were listed.

Primary Analyses

Optimal Concentration: For the Lyral® T.R.U.E. TEST allergen, a determination was made of the lowest concentration that elicited a positive skin reaction (+1 or +2) in 70% to 90% of the sensitive subjects based on the investigator's determination of positive reactions at Visit 5. If a significant number of +3 reactions were elicited, the optimal allergen concentration based on +1 and +2 positive reactions was selected.

Reaction Frequencies: For each allergen and concentration, the number and frequency of subjects with positive, negative, irritant, and doubtful reactions were tabulated at each visit for all enrolled subjects. In all cases, 95% confidence intervals (CIs) also were calculated.

Agreement of Skin Reactions: The number and percent agreement between negative, irritant, doubtful, and positive skin reactions (1+, 2+, and 3+) associated with each dose of the Lyral® T.R.U.E. TEST allergen and the reference allergen were tabulated for Visits 3 and 4 as was the number and percent agreement of skin reactions among the three test doses themselves. Concordance and discordance (with 95% CIs) between the test site skin reactions obtained for each concentration of the Lyral® T.R.U.E. TEST allergen and the test site skin reactions obtained for the reference allergen in petrolatum were calculated based on all enrolled subjects. A similar calculation was performed to evaluate the concordance and discordance among the three doses of the Lyral® T.R.U.E. TEST allergy.

Statistical differences between concentration-related frequencies were evaluated for each dose of the T.R.U.E. TEST allergen using the generalized estimating equations (GEE) approach, which is based on probability distributions. Alternatively, responses from each allergen dose were compared and the agreement was assessed using the kappa statistic. Similar calculations were performed comparing each test dose of the allergen to the reference allergen and to the negative control. Probability values were presented as a result of testing against the null hypothesis that agreement was based on chance alone. Statistical significance was based on a two-sided hypothesis with an inference level of 0.05 or less.

Secondary Analyses

For all subjects combined, the number and frequency of subjects who reported none, weak, moderate, and strong tape irritation and the number and frequency of subjects who reported itching and burning upon patch test removal were tabulated.

As noted above, the frequencies of all patch test reaction scores (positive [+1, +2, and +3], negative, irritant, and doubtful) were calculated for each concentration of Lyral®.

Name of Sponsor/Company:	**Name of Active Ingredient:**	**Name of Finished Product:**
SmartPractice	Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral®)	T.R.U.E. TEST investigational Lyral® allergens

The number and frequency of late and persistent skin reactions were tabulated for all enrolled subjects.

For each allergen concentration, the number and frequency of subjects who reported hyperpigmentation, hypopigmentation, pruritus, or other reactions were tabulated for all enrolled subjects.

The overall number and frequency of subjects who reported an adverse event (AE) and who reported a severe AE were tabulated for all subjects combined. In particular, AEs were listed by subject and serious AEs were described in narratives.

Summary – Conclusions:

Twenty-two subjects were enrolled in and completed the study. At the time of enrollment based on previous patch tests, all 22 of the enrolled subjects were sensitive to Lyral®; 18 subjects were also sensitive to Fragrance mix 2.

Efficacy Results:

A summary of the efficacy (i.e., performance) results follows.

- Based on the investigator's determination of positive responses, the greatest proportion of subjects in the PP population who had a positive reaction to Lyral® on the T.R.U.E. TEST panel was 70% at the highest concentration of 0.40 mg/cm^2. Thus, this was the only concentration of the test allergen that elicited a positive response from 70% or more of the sensitive subjects to meet the criteria for the optimal dose.
- In contrast, the reference allergen of Lyral® 5% in petrolatum elicited 91% positive reactions (20 of 22 responses were positive in the ITT safety population). Hence, the reference allergen was more efficacious than the T.R.U.E. TEST doses of allergen in eliciting positive reactions.
- For the ITT safety population across visits, the Cohen's Kappa values ranged from 0.313 to 0.466, indicating fair to moderate agreement between the Lyral® doses and the reference allergen in petrolatum. The agreement between the T.R.U.E. TEST doses of allergen and the reference allergen was also fair to moderate based on the investigator's determination of positive reactions.
- When the the Lyral® doses were compared to each other across visits, the values of Kappa ranged from 0.567 -0.820 indicating moderate to very good agreement. In other words, the different doses were associated with similar results. When the Lyral® doses were compared to each other in terms of the investigator's determination of positive reactions, the concordance increased, ranging from 0.718-0.908 and indicating substantial agreement among the doses.
- For the ITT safety population at Visits 3 and 4, the GEE analysis indicated a significant overall difference between treatments. The results of the multiple comparison analysis indicated a difference between the Lyral® doses 0.40, 0.20, and 0.10 mg/cm^2 and the negative control. Additionally, the Lyral® 0.40 dose was statistically different than the Lyral®0.10 dose.
- Based on the investigator's determination of positive reactions, the GEE analysis indicated a significant overall difference between treatments. The results of the multiple comparisons analysis indicated a difference between the Lyral® doses (0.40, 0.20, and 0.10 mg/cm$^{2)}$ and the negative control.

Name of Sponsor/Company:	**Name of Active Ingredient:**	**Name of Finished Product:**
SmartPractice	Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral®)	T.R.U.E. TEST investigational Lyral® allergens

Safety Results:

The evaluation of the safety of three concentrations of Lyral® within T.R.U.E. TEST patches is summarized below:

- There were no serious or severe AEs reported during the study, and none of the 22 enrolled subjects discontinued the study because of an AE.
- Three AEs were reported during the study, all of which were considered mild. There was one case of conjunctivitis, which was considered unrelated to the test protocol. There were two cases of pruritis involving an application site, one of which was considered possibly related to treatment and one of which was not considered related to treatment. In all three cases the AE resolved.
- No patient had a late reaction to any of the patch tests. Overall, 18 of the 22 subjects (81.8%) had a persistent skin reaction to at least one of the allergen test doses or to the reference allergen or to both. More subjects had persistent reactions to the reference allergen than to the test doses of the allergen.
- No patient experienced hypo- or hyperpigmentation, and the overall frequency of pruritis was low.
- The majority of subjects experienced no or weak tape irritation in association with the test panel and the reference allergen.
- The majority of subjects experienced no or weak itching burning in association with the test panel and the reference allergen.
- Overall, based on review of the AEs, tape-induced reactions, burning and itching reactions upon patch removal, and late and persistent skin reactions, no safety signals or trends appear to have been associated with the allergen formulations or control patches.

Conclusions:

Overall, the results of this study indicate that the reference allergen in petrolatum was more efficacious in eliciting positive skin reactions than the Lyral® T.R.U.E. TEST allergen. Based on the investigator's determination of positive responses, the greatest proportion of subjects in the PP population who had a positive reaction to Lyral® on the T.R.U.E. TEST panel was 70% at the highest concentration of 0.40 mg/cm^2. Thus, this was the only concentration of the test allergen that elicited a positive response from 70% or more of the sensitive subjects to meet the criteria for the optimal dose. Based on a review of AEs, the frequency of patch test reaction scores, the evaluations of irritation, burning, and stinging, the evaluations of late and persistent skin reactions, and the reports of hyperpigmentation, hypopigmentation, pruritus, and other reactions, the safety results indicate that the Lyral® T.R.U.E. TEST allergen was well tolerated at all concentrations when applied under occlusion for two days.

3. TABLE OF CONTENTS

3.1 In-Text Tables

3.2 Figures

3.3 Listings (link to listings document)

4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
CI	Confidence Interval
CRF	Case Report Form
CV	*Curriculum Vitae*
FN	False Negative
FP	False Positive
GCP	Good Clinical Practice
GEE	Generalized Estimating Equations
HIPAA	Health Insurance Portability and Accountability Act
ICDRG	International Contact Dermatitis Research Group
IEC	Independent Ethics Committee
IRB	Institutional Review Board
PVP	Polyvinylpyrrolidone
SAS	Statistical software from SAS Institute Inc., Cary, NC
STD	Standard Deviation
T.R.U.E. Test	Thin-layer Rapid Use Epicutaneous Test
TN	True Negative
TP	True Positive
US	United States

5. ETHICS

5.1 Independent Ethics Committee or Institutional Review Board

Prior subjects at the investigational site were screened, The study protocol, the subject information/consent form, the Thin-layer Rapid Use Epicutaneous (T.R.U.E.) Test Package Insert, all known safety information, each investigator's *curriculum vitae* (CV), and any advertising materials used to recruit subjects were submitted to The Research Ethics Committee of the Region of Southern Denmark. The IRB approved all required documents; a copy of the approval letter was provided to SmartPractice. Information regarding the specific IRB utilized in this study is appended (Appendix 16.1.3).

5.2 Ethical Conduct of Study

This study was conducted in accordance with Good Clinical Practices (GCP) and the ethical principles that have their origins in the Declaration of Helsinki.

5.3 Subject Information and Consent

Before subjects were enrolled in the study and before any study-specific assessments were conducted, subjects who were willing to participate in the study read, signed, and dated an informed consent document; a sample of the consent document is appended (Appendix 16.1.3). The informed consent provided subjects with information regarding the purpose, procedures, requirements, and restrictions of the study along with any known risks and potential benefits associated with the study drug, the available compensation, and the established provisions for maintaining confidentiality of personal protected health information. Subjects also were informed about the voluntary nature of participation in the study and whom to contact with questions or concerns. The investigator kept the original signed version of the consent and provided a duplicate to each subject.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

6.1 Investigators

One investigator and one sub-investigator in Denmark were recruited to participate in this clinical study. The investigators were selected based on their experience performing and evaluating topical allergy skin tests, their willingness to conduct the study according to GCP requirements, and their ability to enter eligible subjects. The *curricula vitae* for the principal investigators are appended (Appendix 16.1.4).

6.2 Contract Research Organizations

Partial obligations for the study were transferred to the following contract research organization:

Data Management	Cliantha Research Limited Opposite Pushparaj Towers, Near Judges Bungalows, Bodakedev Ahmedabad-380 054 Gujarat, India Telephone: +91-79-2685 3088-92
Statistical Analysis	James P. Bowman & Associates, LLC 6409 Roth Ridge Dr. Loveland, OH 45140 Telephone: (513) 340-1003

Authors
The authors of this report, along with their functions and affiliations are as follows:

Jim P. Bowman	Biostatistics	James P. Bowman & Associates, LLC
Shelley A. Kick, Ph.D.	Medical Writing	SmartPractice 3400 E.McDowell Rd. Phoenix, Arizona 85008-7899 Telephone: 602-225-0595

7. INTRODUCTION

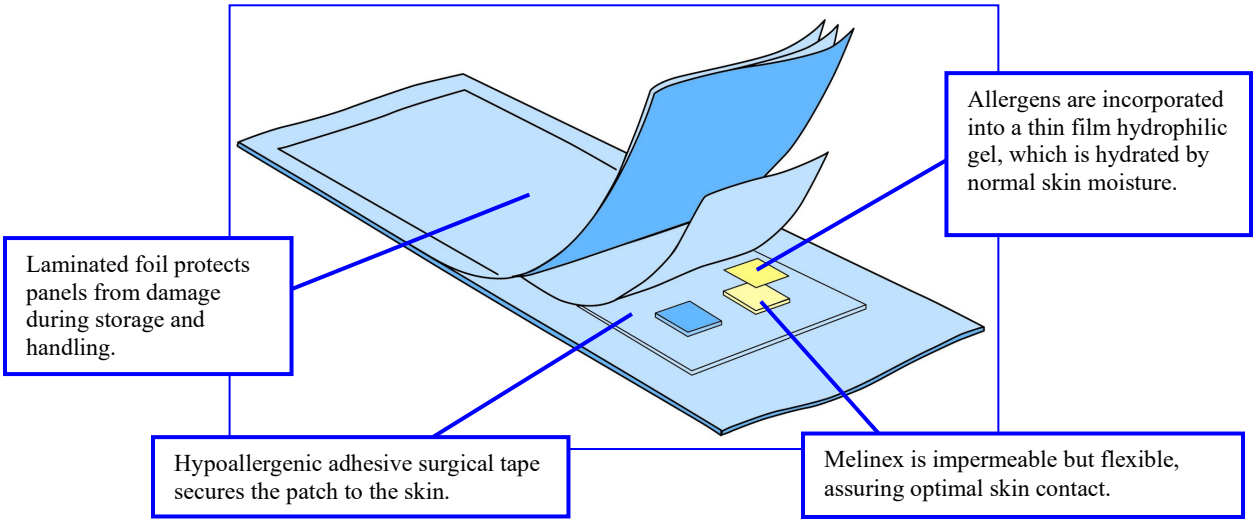
T.R.U.E. TEST, (Thin-layer Rapid Use Epicutatneous Patch Test) was originally granted a Biologics License for 23 allergens and a blank patch (control) in 1994 (BL No. 1623). The allergens were selected from substances widely reported to induce allergic contact dermatitis (ACD). As new allergens become clinically relevant, there is an ever-growing need to expand the number of allergens included in T.R.U.E. TEST. The next 5 allergens were added to a third panel of the T.R.U.E. TEST product in 2007 (BL103738/5019 and BL 103738/5027). An additional seven allergens were added in 2012. The current U.S.-available T.R.U.E. TEST product includes three panels of 36 allergen polyester patches including a blank patch.

Each 0.81 cm^2 allergen patch is coated with one specific allergen or allergen mix. The allergens/allergen mixes are incorporated in exact dosage in a hydrophilic gel. The allergen-gel preparation is coated on an impermeable backing of polyester and dried to a thin film. The coated sheet is then cut into 9 mm x 9 mm squares (test patches). These allergen gel preparations are attached to a nonwoven rayon fiber tape coated with a medical acrylic adhesive (see Figure 7-1) to form a standard test panel. The 3 panels together form a standard test kit.

All 3 panels are typically applied to the skin of the upper back. The humidity of the skin hydrates the film and transforms to a gel, allowing the allergen to migrate into the skin, thereby reaching the cells of the immune system. The test is removed after 48 hours and read 72-96 hours after the application when the allergic responses are fully developed and any mild irritant reactions have faded. Additional readings at 1 week and 21 days after panel placement are also advised in some cases.

As a ready-to-use patch test method, T.R.U.E. TEST is designed for use by licensed physicians in the diagnosis of ACD. T.R.U.E. TEST has been evaluated in several large, multi-center clinical studies and is the only combined allergen and patch panel/chamber product currently approved by the Food and Drug Administration (FDA) in the United States.

Figure 7-1: Illustration of the T.R.U.E. TEST Product



Physicians sometimes test patients with their own combination of allergens, and/or patient-supplied products. Currently in the US, several patch test chambers are licensed for use as medical devices, including Finn Chambers®, allergEAZE® Patch Test Chambers, and IQ® Chambers. For each of these devices, the patch test chambers must be filled with a specific allergen prior to patient application. These contact allergens typically are obtained from one of several European manufacturers, depending upon the physician's preference. They are prepared in petrolatum (or aqueous base), or alternatively, in a special excipient intended to promote solubilization or dispersion. Typically, each allergen is supplied at a single concentration; these test concentrations can range from 0.002% to 50% (based on weight) depending on the specific allergen and vehicle/excipient used.

To date, petrolatum allergens are considered the standard of care for ACD patch testing. However, allergen composition, test conditions, patient sensitivity, and physician experience can influence the intensity and reading of patch test reactions [1, 2, 3]. Therefore, it is not surprising that concordance between T.R.U.E. TEST and petrolatum allergens reportedly averages around 64% but may range from 18% to 81% [4, 5]. By comparison, within-patient patch testing with T.R.U.E. TEST allergens has been associated with concordance in 95% of 435 positive patch test reactions [6].

To continue to improve the efficiency of T.R.U.E. TEST in diagnostic ACD, it is important to expand the number of test allergens as new allergens become clinically relevant. Consequently, the current study was designed to evaluate the diagnostic performance of three different concentrations of Lyral® T.R.U.E. TEST allergens in 20 adult subjects with clinical histories of contact dermatitis and positive patch test results to Lyral® or Fragrance mix 2.

8. STUDY OBJECTIVES

The primary objective of the study was to determine the lowest concentration of ascending doses (0.40 mg/cm^2, 0.20 mg/cm^2, and 0.10 mg/cm^2) of 3-cyclohexene carboxaldehyde (Lyral®) in a Thin-layer Rapid Use Epicutaneous (T.R.U.E.) Test panel to elicit +1 or +2 positive reactions in 70-90% of 20 adult "sensitive" subjects with a clinical history of contact dermatitis and a positive patch test (historical) to Lyral® or Fragrance mix 2.

The secondary objective of the study was to evaluate the safety of Lyral® in 20 adult "sensitive" subjects with a clinical history of contact dermatitis and a positive patch test (historical) to either Lyral® or Fragrance mix 2.

9. INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan: Description

This was a prospective, single-center, double-blind, randomized, phase I clinical designed to compare the diagnostic performance and safety of different concentrations of Lyral® T.R.U.E. TEST allergen in 20 adult subjects with clinical histories of contact dermatitis and a positive patch test (current or previous) to Lyral® in petrolatum or to Fragrance mix 2.

At the first study visit, potential subjects provided their written consent, medical histories, and concomitant medication usages and were screened against the inclusion and exclusion criteria.

At Day 0 (Visit 1), female subjects underwent urine pregnancy tests. Current dermatitis sites were examined in all eligible subjects. All eligible subjects then were randomized to receive one of three patch panels. Each panel contained three concentrations of the allergen in polyvinylpyrrolidone (PVP) and one negative control (PVP only); the only difference between panles was in the configuration (i.e., specific location of the different concentrations) of the allergens in the individual patches on the panels. Subjects had a randomized panel applied to their back, along with a patch test chamber that contained 20 mg of Lyral®, 5%, in petrolatum.

The panels with the patch test chambers were removed 2 days later at Visit 2. During this visit, the integrity of the test panels was assessed. After the patch tests were removed, the skin was allowed to rest for 20 minutes. All test site skin reactions were then evaluated along with any tape irritations. Subject reports of itching and/or burning at the test sites also were recorded.

Additional evaluations of test site skin reactions were conducted at 4 days (-1 day) (Visit 3), 7 days + 1 day (Visit 4), and 21 days ± 2 days (Visit 5) after the initial placement of the patch panels. At each of these evaluations, adverse events (AEs) were documented. At Visits 4 and 5, late-phase and/or persistent skin reactions were recorded. All subjects exited the study at the completion of Visit 5, which could have been conducted over the telephone.

9.2 Discussion of Study Design, Including the Choice of Control Groups

This study was designed to evaluate the diagnostic performance of three different concentrations of Lyral® T.R.U.E. TEST allergens in 20 adult subjects with clinical histories of contact dermatitis and a positive patch test to Lyral® in petrolatum or to Fragrance mix 2.

The study employed a T.R.U.E. TEST panel with different concentrations of the allergen formulated in PVP. To ensure accurate assessments of allergy test outcomes, a negative control patch that contained only PVP was included on the panel. The reference allergen (20 mg of Lyral®, 5%, in petrolatum) was dispensed in a commercially available patch chosen at the investigator’s discretion.

True “disease” status (i.e., ACD to a specific allergen) is rarely known definitively. Clinically, patch test results, clinical histories, observed symptoms, and provocation tests (if performed) are used to report the “disease” status of a patient, and discordance among these diagnostic parameters is common. Moreover, symptoms and test results can change over time, presumably reflecting decreased exposure or variability in a patient’s skin condition or overall health. These factors contribute to the difficulty of assigning a positive “disease” status (i.e., “sensitive” status) to patients.

To minimize such variability, only subjects with a clinical history of contact dermatitis and a positive patch test (historical) to either Lyral® or Fragrance mix 2 were included in the study. The reference standard containing Lyral® in petrolatum was applied to the subjects using standard, commercially available patch test chambers to confirm their reactions to the test concentrations of the allergen.

The methods of evaluating the allergen concentrations in this study were comparable to those used in the evaluation of the T.R.U.E TEST patches in general. Specifically, a 2-day occlusion period followed by an evaluation period lasting 19 days (21 days after initial patch application) was considered sufficient for evaluating the concentrations. All skin reactions were evaluated using standard patch testing guidelines established by the International Contact Dermatitis Research Group (ICDRG).

Analyses were based on two populations: the intent-to-treat (ITT) safety population composed of all subjects who received a patch test (N = 22) and the per protocol (PP) population composed of subjects who completed the protocol with no major protocol violations (N = 20). The ITT safety population was used to evaluate the safety endpoints and to support efficacy endpoints. The PP population was used to evaluate the primary study endpoints.

Both the principal and subinvestigator used identical scales and received similar training. Throughout the study, subjects were prohibited from participating in activities or from using concomitant medications/therapies that could have interfered with the adherence of the patch applications or with the assessment of the test sites. To help minimize potential bias, the order of the T.R.U.E. TEST patches and negative control were randomized in a double-blind fashion such that neither the subject nor the evaluator knew which test patch corresponded to which allergen concentration. Specifically, subjects received one of three differently configured patches; the only difference between patches was the specific location of the negative control and each of the allergen concentrations. In all cases, however, all subjects were exposed to a T.R.U.E. TEST panel with three concentrations of Lyral® in PVP and the negative control as well as to the reference allergen on a separate panel. The identity of the reference allergen could not be blinded.

Finally, the study population was planned to include a substantial proportion of female subjects (at least 50% but no more than 85%). This latter requirement allowed for extrapolation of the

study results to the general population of dermatitis sufferers who might be evaluated by physicians using the T.R.U.E. TEST product.

9.3 Selection of Study Population

The study population was planned to include 20 adult subjects, 18 years of age and older, who were in generally good health. Subjects must have had current or previous symptoms and/or histories that were consistent with ACD and must have had a positive patch test reaction to Lyral® or Fragrance mix 2. Twenty-two subjects were enrolled in the study, all of whom were sensitive (i.e., had positive patch test reactions) to Lyral® or Fragrance mix 2. In general, the enrollment sought to include at least 50% (but not more than 85%) female subjects.

9.3.1 Inclusion Criteria

To be included in the study, all of the following criteria must have been met:

1. Males and females 18 years of age or older.
2. Clinical history of contact dermatitis and positive patch test (current or previous) to either Lyral® or Fragrance mix 2; otherwise, in good general health.
3. Unable to become pregnant or willing to use an acceptable method of contraception to prvent pregnancy if female of childbearing age:
    a. Inability to become pregnant would include all male subjects and female subjects who were postmenopausal for at least 1 year or who were surgically sterile (had had a hysterectomy, bilateral ovariectomy, uterine ablation or bilateral tubal ligation).
    b. Acceptable methods of contraception included (i) systemic birth control (the same type of birth control for at least 3 months before entering the study and continuation of this type of birth contrl throughout the study), (ii) double-barrier methods (condom with spermicide or diaphragm with spermicide, (iii) intrauterine device (IUD), (iv) vasectomized partner, or (v) abstinence from sexual intercourse.
4. Have read and signed the consent form and are able to fulfill the study requirements and make all required visits.

9.3.2 Exclusion Criteria

To be included in the study, none of the following criteria could have been met:

1. Lactation or pregnancy, determined by urine pregnancy test (UPT) for females of childbearing potential. UPT must be conducted before patch placement.
2. Treatment with topical corticosteroids on or near the test area during the previous 7 days.
3. Treatment with systemic corticosteroids or immunosuppressives during the previous 7 days (inhaled treatments were permitted).
4. Treatment with ultraviolet light (UV) light, including tanning, during the previous 3 weeks.
5. Acute dermatitis outbreak or dermatitis on or near the test area on the back.
6. Participation in another clinical study involving an investigational drug, treatment or device currently or within the previous 3 weeks.
7. Unable to comply with activity restrictions (e.g., protecting test panels from excess moisture due to showering or vigorous activity).
8. Unable or unwilling to comply with multiple return visits.

9.3.3 Removal of Subjects from Therapy or Assessment

Subjects were permitted to withdraw from the study at any time. However, subjects were not necessarily withdrawn from the study for missing scheduled visits. Subjects were contacted and outcomes were documented as described in the clinical protocol and investigator brochure. If subjects missed two or more clinic visits, they could be withdrawn from the study at the discretion of the local investigator.

The investigator could withdraw subjects if deemed medically necessary or if the subject could no longer meet study requirements. Reasons for subject withdrawal were to be described and recorded in the subjects' CRF.

Additional criteria for subject withdrawal included the following:

1. Overreactions, defined as a response to a particular patch greater than Extreme Positive (3+). In such cases, test panels were to be removed immediately and reactions treated per standard medical guidelines.

2. Unacceptable adverse events such as the development of severe reactions, dermatitis flare-up, or other adverse events that may be considered unacceptable.

Patients were informed that withdrawal from the study would not impact their future care at affiliated hospitals or clinics or other health care facilities involved in this study

9.4 Treatments

9.4.1 Treatments Administered

The T.R.U.E. TEST patch was applied to the back of each subject as was the reference allergen (Lyral® 5% in petrolatum) dispensed in a standard patch chamber. Each T.R.U.E. TEST patch

consisted of one panel that included the following concentrations of the allergen in one of three configurations:

- Lyral® in PVP, 0.10 mg/cm^2
- Lyral® in PVP, 0.20 mg/cm^2
- Lyral® in PVP, 0.40 mg/cm^2
- PVP (negative control), 0.0 mg/cm^2

In addition to their standard means of application, the panels with the test allergens (applied using T.R.U.E TEST patches) and the reference product (applied using patch chambers) were further secured with medical tape to minimize the possibility of loosening due to inadvertent subject activity and/or moisture accumulation.

At Visit 1 subjects were randomized to receive one of the three differently configured T.R.U.E. TEST panels. All three configurations included all three test allergen concentrations and the negative control. The only difference between the patches related to the specific location of each allergen concentration and the negative control. Thus, neither the subjects nor the evaluators knew which panel corresponded to which test product. However, because there was only one reference allergen, its identity could not be blinded.

9.4.2 Identity of Investigational Product(s)

The investigational T.R.U.E. TEST patches were packed and labeled by SmartPractice Denmark ApS. To protect against light and air, the patches were sealed in opaque, aluminum foil pouches. The products were labeled with allergen batch codes and expiration dates.

The reference product, Lyral® 5%, in petrolatum, was prepared at the Department of Occupation and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden. The allergen was applied at the recommended dosage of 20 mg per chamber. Subjects and investigators were not blinded to the location and order of this reference allergen. The reference allergen was to be stored at room temperature or under refrigeration, per the specific storage requirements specified on the product label.

The batch number for the test products used in this study is listed in Table 9.4.2-1.

Table 9.4.2-1: Batch Numbers

Test Product	Batch Number
Lyral® in PVP, 0.10 mg/cm^2	13001
Lyral® in PVP, 0.20 mg/cm^2	13001
Lyral® in PVP, 0.40 mg/cm^2	13001
PVP (negative control), 0.0 mg/cm^2	13001

9.4.3 Method of Assigning Subjects to Treatment Groups

Designated staff members at each site dispensed the patches by assigning subject numbers in an ascending numerical order using the lowest available number. Treatments were assigned to subjects at Visit 1 according to a predetermined, random schedule that was based on subject number. Access to the randomization schedule was kept restricted until after the database was locked and the study was unblinded; see Appendix 16.1.7.

Subjects received one of the three T.R.U.E. TEST patches and were exposed to each of the three concentrations of Lyral® and the negative control. The only difference between randomized patches was the specific location of the allergen concentrations and the negative control. In addition to the T.R.U.E. TEST patch, all subjects were exposed to the reference allergen in petrolatum; the reference product was applied using standard patch test chambers.

9.4.4 Selection of Doses in the Study

9.4.4.1 Lyral®

Fragrances are a common cause of ACD, and Lyral® (hydroxyisohexyl 3-cyclohexene carboxaldehyde) is a synthetic fragrance--an aldehyde that is lipophilic enough to penetrate the skin. It is used in more than 50% of marketed deodorants as well as in many perfumes and other personal care products.[7] Although Lyral® has been used as a fragrance in the cosmetic industry for decades, it has only recently been identified as an allergen.[7,8] In 2003 the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) determined that consumers should be informed about the presence of fragrance ingredients with a well-recognized sensitizing potential when used in cosmetic products. Lyral® was one of 13 such fragrance chemicals. Lyral® is also included on the North American Standard Series of patch test allergens.[9]

Case reports and large patch test studies have provided evidence of Lyral® sensitivity in the population, especially among patients with a fragrance or cosmetic allergy. Studies from various international centers have reported contact allergy rates ranging between 1.5% and 7.3% for Lyral® 5%, in petrolatum tested in consecutive patients.[7,10,11,12] Fragrance mix 2, which contains 6 common chemicals, is reported to be a useful marker of fragrance allergy. It has been

associated with contact allergy rates as high as 5% in various national baseline patch test series.[13] Of the individual fragrance chemicals included in Fragrance mix 2, Lyral® has been the most common sensitizer: 43.2% of individuals testing positive to Fragrance mix 2 have been allergic to Lyral®.

Based on its prevalence as a sensitizer, Lyral® has been included as part of the baseline series of allergens recommended by many international research groups.[10,11,14,15] The recommended dosage is 5% w/w (2.0 mg/cm^2) in petrolatum, which corresponds to 0.20 mg/cm^2 (0.16 mg/patch) in T.R.U.E. TEST. This dose reponse study included one concentration below and one concentration above the expected label: 0.40, 0.20, and 0.10 mg/cm^2.

9.4.5 Selection and Timing of Dose for Each Subject

The T.R.U.E. TEST patches were applied to the back of every subject. The subjects were instructed to keep the test sites dry and protected. After two days of occluded exposure, the patches were removed from all subjects. After the patches were removed, subjects were instructed to continue to avoid activities that involved excess moisture (sweat or water), movement, or sun exposure until they had completed their final study visit.

9.4.6 Blinding

During Visit 1, designated personnel at the study site assigned and applied the blinded patches to eligible subjects in numerical sequence. Neither the subjects nor the investigators were aware of the placement of the allergen concentrations or the negative control on the T.R.U.E. TEST patch. A sealed envelope containing the location and identity of each test allergen concentration on the T.R.U.E. TEST patch was provided to each investigator for every subject. In case of a medical emergency, the investigator was allowed to open the envelope; investigators were encouraged to contact the Sponsor's medical monitor before opening any envelop if there was sufficient time. Regardless of prior notification, the investigator was instructed to inform the Sponsor of any instances in which the treatment code was broken and the reasons for such instances. During the course of this study, the treatment code was not broken for any subject. Because only one reference allergen was used, it was not possible to blind its identity.

9.4.7 Prior and Concomitant Therapy

Subjects were excluded from the study if they had undergone topical treatment with corticosteroids or other immunosuppressive agents on or near the test area during the 7 days prior to screening. Subjects also were excluded if, during this same time period, they had undergone systemic treatment with corticosteroids (equivalent to >10 mg prednisone) or other immunosuppressive agents. Finally, subjects must not have used ultraviolet light treatments (including tanning) during the 3 weeks before screening.

Although not related to concomitant therapy, subjects had their activities restricted during the study. Specifically, during the first 2 days of the study, subjects must have avoided activities that could have hindered patch adherence or interfered with skin reaction evaluations. This restriction included activities that involved excess moisture (sweat or water), movement, or sun exposure. After the patches were removed, subjects must have protected the test sites from sun, irritation, medicaments, and foreign or harsh substances until their final study visit was completed.

9.4.8 Treatment Compliance

The investigators accounted for all investigational products used in this study. Specifically, the investigators maintained records of test and reference product receipt, dispensation, and return. Any discrepancy in the records was documented. The T.R.U.E. TEST patches were stored between 36°F (2°C) and 46°F (8°C), inclusively. The reference allergen was stored at room temperature or under refrigeration per the manufacturer's requirements.

Adhesion of the patches was documented upon their removal at Visit 2. Specifically, the investigators reported the degree of adhesion using the following scale:

> *Excellent*: skin contact good; all tape edges adherent; all allergens in contact with the skin
> *Good*: skin contact acceptable; some tape edges lifting; all allergens in conact with the skin
> *Poor*: little to no skin-to-panel contact with panel; one or more allergens not in contact with the skin
> *Detached*: panel completely off the skin; none of the allergens in contact with the skin

If adhesion was scored as *Poor* or *Detached*, the allergen sites not in contact with skin at the time of panel removal were identified.

Factors that contributed to poor adhesion may have been recorded on the CRF and may have included oily skin, excess hair, wet patch, sweat/skin moisture, and activity-associated failure. Note that subjects were restricted in regard to performing certain activities that could have affected patch adherence (see Section 9.4.7), and subjects who failed to comply with the study requirements could have been discontinued (see Section 9.3.3).

9.5 Efficacy and Safety Variables

9.5.1 Efficacy and Safety Measurements Assessed and Flow Chart

The study evaluation and visit schedule is presented in Table 9.5.1-1; full details regarding the study variables follow the table. Additional information regarding the study methodology also may be found in the protocol; see Appendix 16.1.1.

Table 9.5.1-1: Study Plan

	Visit 1 (Day 0)	Visit 2 (Day 2)	Visit 3 (Day 4 (-1))	Visit 4 (Day 7(+1))	Visit 5[a] (Day 21 (±2))
Informed consent	X				
Privacy authorization (if applicable)	X				
Medical history	X				
Medication history	X				
Inclusion/exclusion criteria	X				
Demographic data	X				
Urine pregnancy test	X				
Dermatitis screen	X				
Panel application	X				
Panel adhesion assessment		X			
Panel removal		X			
20-minute wait before irritation assessment		X			
Tape irritation assessment		X			
Subject-reported itching and burning		X			
Skin reaction assessment and scoring			X	X	X
Determination of late/persistent reactions				X	X
Investigator determination of positive reactions					X
Concomitant medication review	X	X	X	X	X
Adverse event query and documentation		X	X	X	X

Abbreviations: HIPAA = Health Insurance Portability and Accountability Act; Screen = screening visit
[a] May have been conducted *via* telephone at the investigator's discretion.

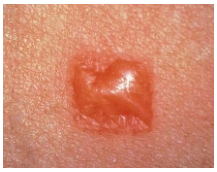
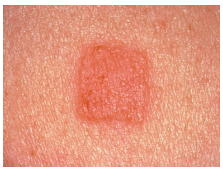
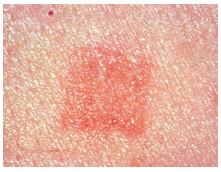
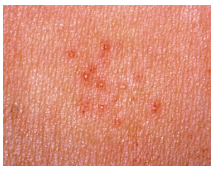
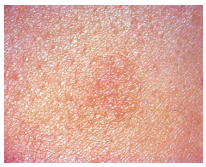
9.5.1.1 Efficacy Variables

The efficacy variables (considered the primary endpoints) collected in this study included skin sensitivity reactions to each of the test concentrations (i.e., Lyral® in PVP as well as the negative control [PVP] applied as a T.R.U.E. TEST panel) and skin sensitivity reactions to the reference product (i.e., Lyral® 5%, in petrolatum, applied using standard patch test chambers). The skin sensitivity evaluations were conducted at Visits 2 (after patch removal) through 5.

All skin reactions were evaluated using standard patch testing guidelines established by the ICDRG. These evaluations considered the presence of erythema, infiltration, papules, discrete vesicles, and bullous reactions. Specifically, the skin reactions were scored as negative (-),

irritant reaction (IR), doubtful reaction (?), weak positive (1+), strong positive (2+), or extreme positive (3+). Complete definitions of each score, along with representative depictions of the corresponding reactions, are presented in Figure 9.5.1.1-1. For the purpose of analysis, a positive reaction was defined as a positive response (weak, strong, or extreme), as determined by the investigator, at Visit 3, Visit 4, or Visit 5.

Figure 9.5.1.1-1: Skin Reaction Scoring Guidelines

Extreme positive (+++)	Strong positive (++)	Weak positive (+)	Irritant (IR)	Doubtful (?)
				
Coalescing vesicles, bullous reaction	Erythema, papules, infiltration, discrete vesicles	Erythema, infiltration, discrete papules	Discrete, patchy, follicular, or homogenous erythema with no infiltration	Faint macular or homogenous erythema with no infiltration

9.5.1.2 Safety Variables

The safety assessments (considered secondary endpoints) included tape-induced irritation and subjects' reports of itching and burning after patch removal at Visit 2, as well as evidence of late and/or persistent reactions. Separate from these evaluations, skin reaction symptoms (including assessments of erythema, infiltration, hyperpigmentation, hypopigmentation, pruritus, and other reactions) were recorded. Finally, AEs and serious AEs were assessed and recorded at each post-application study visit. A description of the methods used for conducting the safety assessments follows.

Tape-Induced Irritation and Associated Itching and Burning

After the patches were removed at Visit 2, the investigators evaluated tape-induced irritation, and the subjects reported their itching and burning. Tape irritation was reported as follows:

- None: no tape irritation
- Weak: faint-to-definite pink erythema
- Moderate: moderate erythema, definite redness
- Strong: Severe erythema, very intense redness

Itching and burning were reported as follows:

- None: no discomfort
- Weak: minimal discomfort
- Moderate: definite discomfort
- Strong: Significantly bothersome; possible interference with sleep or daily activity

Late and/or Persistent Reactions

Late reactions were defined as reactions that initially occurred 7-10 days after application of the patches. Persistent reactions were defined as positive test site skin reactions that first appeared at Day 2 and/or Day 3 and/or Day 4 and persisted through Day 7 or to Day 21. Persistent reactions were further clarified as persistent/healing or persistent/escalating.

Skin Reactions Symptoms

Investigator assessments of erythema, infiltration, hyperpigmentation, hypopigmentation, pruritus, and other reactions were recorded at Visit 5. For each of these variables, observations were reported as none, mild, moderate, or severe.

Adverse Events and Serious Adverse Events

In this study, an AE was defined as any untoward medical occurrence in a study subject associated with the use of a drug in humans, whether or not considered related. An adverse reaction was defined as any adverse event caused by a drug.

AEs relevant to this study included all suspected adverse reactions to the test tape adhesive and/or allergen patches; reactions such as overdose, sensitivity, or toxicity; apparently unrelated illnesses, including the worsening of a pre-existing illness; injury or accidents; and abnormalities in physiological testing or physical examination that required clinical intervention. AEs also included dermatitis flare-ups, a worsening of existing dermatitis symptoms, or new outbreaks of dermatitis.

Serious AEs may have involved one or more of the following: death; a life-threatening AE; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions; a congenital anomaly/birth defect; or another important medical event that required medical or surgical intervention to prevent death, a life-threatening condition, or hospitalization.

Regardless of whether the event was serious or nonserious, the investigators assessed the severity of the events according to the following criteria:

- Mild: minimal symptoms / annoying / minimal discomfort. Did not interfere with the subject’s usual function.

- Moderate: definite discomfort / requires medication for relief. Interfered to some extent with subject's usual function.
- Severe: symptoms interfered significantly with the subject's usual function (daily activity/sleep).

All AE reporting began following patch application at Visit 1 and ended at the last study visit (Visit 5). All serious AEs, along with those nonserious AEs that were considered by the investigator to be possibly related to the investigational product, were followed until they resolved or until the investigator determined that they were "chronic" or "stable." Resolution and follow up of AEs were documented on subjects' CRF.

9.5.2 Appropriateness of Measurements

The methods used in this study to evaluate the test concentrations of the allergen were similar to those used in the development of T.R.U.E. TEST. Including both a negative control and a reference standard applied to all subjects ensures the robustness of the evaluations. Furthermore, the use of both objective (investigator-assessed) and subjective (subject-assessed) evaluations provides an accurate evaluation of the concordance and discordance of the allergen concentrations and the reference allergen. Finally, all skin reactions were evaluated using standard patch testing guidelines established by the ICDRG.

9.5.3 Primary Efficacy Variable(s)

The primary endpoints (i.e., the endpoints used to evaluate the diagnostic performance of the Lyral® T.R.U.E. TEST allergens) included the following:

- Determination of the lowest allergen concentration that elicited a positive skin reaction (+1 or +2) to Lyral® in 70% to 90% of the sensitive subjects (i.e., all subjects by definition). A positive reaction was defined as a positive test site skin reaction, as determined by the investigator, observed at Visit 3, Visit 4, or Visit 5.
- Frequency of positive, negative, doubtful, and irritant reactions for each allergen at the tested concentrations.
- Concordance and discordance between each of the concentrations of Lyral® T.R.U.E. TEST and the reference allergen in petrolatum.

9.5.4 Drug Concentration Measurements

This study was designed to evaluate the diagnostic performance and safety of three concentrations of Lyral® (in PVP at concentrations of 0.10, 0.20, and 0.40 mg/cm^2). The primary endpoints consisted of the variables used to evaluate diagnostic performance, while the secondary endpoints consisted of the variables used to evaluate safety. A summary of the

primary endpoints is included in Section 9.5.3. The secondary endpoints consisted of the following:

- Tape irritation at patch test removal.
- Incomplete panel adhesion.
- Itching and burning at patch test removal.
- Late-phase reactions.
- Persistent reactions.
- Erythema, infiltration, hyperpigmentation, hypopigmentation, pruritus, or other reactions.
- Other AEs.
- Severe AEs.

9.6 Data Quality Assurance

Monitoring visits to the sites were made periodically to assess compliance with current GCP guidelines, to evaluate clinical trial progress, to assess the continued acceptance of the clinical trial site facilities, and to verify the data recorded on the CRFs. The final monitoring visits took place after the last subject exited the study (monitoring close-out visit).

Subject safety was ensured by noting that appropriate consent documents existed, that appropriate study procedures were followed, and that AEs were reported and followed as appropriate. The investigators maintained detailed records on all study subjects; study-specific data were recorded onto source document/case report forms (CRFs). A 100% verification of the data was performed. The source documents were scanned into each subject's electronic data file by the investigational site, and the originals were collected and remain at SmartPractice (Phoenix).

Medical monitoring processes and procedures were instituted to ensure that all medical data were valid and reliable. The procedures were designed to provide early recognition, identification, and reporting of issues that could have affected the health and well-being of the subjects. The designated medical monitor's responsibilities included providing requested clarification to sites regarding protocol procedures, querying protocol violations/deviations, asking for clarification or further information related to reported safety events and other clinical data, and unblinding treatment in case of a medical emergency.

9.7 Statistical Methods Planned in the Protocol and Determination of Sample Size

9.7.1 Statistical and Analytical Plans

A summary of the statistical analysis plans that were developed and implemented prior to database lock and breaking of the study blind for treatment assignment is presented below. This summary includes descriptions of study populations and the planned analyses for the primary (diagnostic performance) and secondary (safety) endpoints. All statistical processing was

performed using SAS software and no imputations were made for missing data. The statistical analysis plan is appended (see Appendix 16.1.9).

Throughout the analyses, patch test reactions were evaluated for all enrolled subjects (N = 22). In this study, “sensitive subjects” included subjects who had had a historical positive patch test reaction to Lyral® and/or Fragrance mix 2.

Analyses were based on two populations: the intent-to-treat (ITT) safety population composed of all subjects who received a patch test (N = 22) and the per protocol (PP) population composed of subjects who completed the protocol with no major protocol violations (N = 20). The ITT safety population was used to evaluate the safety endpoints and to support efficacy endpoints. The PP population was used to evaluate the primary study endpoints.

Demographic (age, gender) and baseline characteristics (previous positive patch test results, type of dermatitis, presence of current dermatitis symptoms, and dermatitis symptom areas) were summarized using descriptive statistics for all subjects combined. Quantitative measures were summarized using the number of subjects (N), along with the mean, standard deviation (STD), median, minimum, and maximum. Qualitative measures were summarized using frequency counts and percentages. All medical histories and concomitant medication usages were listed.

9.7.1.1 Primary (Diagnostic Performance) Analyses

Optimal Concentration: For the Lyral® T.R.U.E. TEST allergen, the lowest concentration that elicited a positive skin reaction (+1 or +2) in 70% to 90% of the sensitive subjects was defined as the optimal concentration.

Reaction Frequencies: For the reference allergen, negative control and the three concentrations of the Lyral® T.R.U.E. TEST allergen, the number and frequency of subjects with positive, negative, irritant, and doubtful reactions were tabulated for all subjects at Visits 3, 4 and 5. For Visits 3 and 4, 95% confidence intervals (CIs) also were calculated for the positive reactions for both the ITT safety and PP population. The number and frequency of positive reactions to each dose of the Lyral® T.R.U.E. TEST allergen, reference allergen and negative control as determined by the investigator at Visit 5 were also calculated.

Concordance/Discordance: The number and percent agreement of skin reactions [negative, irritant, doubtful, and positive (1+, 2+, and 3+)] between each of the three doses of the Lyral® T.R.U.E. TEST allergen and the reference allergen were calculated for both the ITT safety and PP populations for Visits 3, 4, and 5 and based on the investigator’s determination of positive reactions.

Concordance and discordance (with 95% CIs) between the test site skin reactions obtained for each concentration of the Lyral® T.R.U.E. TEST allergen and those obtained for the reference allergens in petrolatum were calculated for the ITT safety and the PP population based on Visits 3 and 4 and on the investigator’s determination of positive reactions. Concordance and

discordance of patch test reactions among the three doses of the Lyral® T.R.U.E. TEST allergen were also calculated for both populations based on Visits 3 and 4 and on the investigator's determination of positive reactions.

9.7.1.2 Secondary (Safety) Analyses

The number and frequency of subjects who reported none, weak, moderate, and strong tape irritation and the number and frequency of subjects who reported itching and burning upon removal of the patch test were tabulated.

As stated in the Primary (Diagnostic Performance) Analyses (Section 9.7.1.1), the frequencies of all patch test reaction scores [positive (+1, +2, and +3), negative, irritant, and doubtful] were calculated for each allergen at each concentration. The optimal allergen concentration based on reaction scores was determined using the lowest concentration that elicited a positive test site skin reaction in 70% to 90% of the sensitive subjects. If a significant number of +3 reactions were elicited, the optimal allergen concentration based on +1 and +2 positive reactions was to be selected.

Statistical differences between concentration-related frequencies were evaluated for each allergen using the generalized estimating equations (GEE) approach, which is based on probability distributions. Alternatively, responses from each allergen dose were compared and the agreement was assessed using the kappa statistic. Probability (p) values were also presented as a result of testing against the null hypothesis that agreement was based on chance alone. Statistical significance was based on a two-sided hypothesis with an inference level of 0.05 or less.

The number and frequency of late and persistent skin reactions to the three concentrations of the Lyral® T.R.U.E. TEST allergen, the reference allergen, and the negative control were calculated for the ITT safety population.

For each concentration of the Lyral® T.R.U.E. TEST allergen, the reference allergen, and the negative control, the number and frequency of subjects who reported hyperpigmentation, hypopigmentation, pruritus, or other reactions were tabulated for the ITT safety population for Visit 4.

The overall number and frequency of subjects who reported an AE and who reported a severe AE were calculated for all subjects combined. In particular, AEs were listed by subject and serious AEs were described in narratives.

9.7.2 Determination of Sample Size

At least 20 sensitive subjects per allergen were planned for inclusion in the statistical analyses. Previous studies indicated that this number would be sufficient for determining diagnostic performance and safety. With a sample size of 20 subjects, the study had a sufficiently high

power to detect a significant difference between the reactions at the lowest and highest doses using the generalized estimation equation (GEE) or kappa approaches as described above.

9.8 Changes in the Conduct of the Study or Planned Analyses

Not applicable; there were no changes to the conduct of the study or the planned analyses. The original protocol is appended (see Appendix 16.1.1).

10. STUDY SUBJECTS

10.1 Disposition of Subjects

Twenty-two subjects were enrolled at the investigational site in Denmark. The first subject entered the study on 18 February 2013 and the last subject exited the study on 17 June 2013. All 22 of the enrolled subjects were sensitive to Lyral®; 18 were also sensitive to Fragrance mix 2. All 22 subjects completed the study.

10.2 Protocol Deviations

At the prinicipal investigator's discretion, two subjects (Subjects 107 and 122) were enrolled into the study despite taking oral methotrexate (15 mg/week) for preexisting eczema in violation of the third exlusion criterion. Both subjects completed the study but were only included in the safety analysis. No other protocol deviations were reported during the study (see Listing 16.2.11).

11. EFFICACY EVALUATION

11.1 Data Sets Analyzed

All computations and tabulations related to efficacy were conducted using the 20 subjects who met all inclusion and exclusion criteria. In this study, “sensitive subjects” included subjects with a clinical history of contact dermatitis and a positive patch test (current or previous) to Lyral® or Fragrance mix 2. Of the 22 enrolled subjects, all were sensitive to Lyral®, but the two subjects (Subjects 107 and 122) entered at the investigator’s discretion in violation of the third exclusion criterion were not included in the efficacy evaluation.

11.2 Demographic and Other Baseline Characteristics

The 22 enrolled subjects had a mean (STD) age of 49.64 (11.78) years and were primarily female (77.3%) and Scandinavian (95.5%). As defined by the inclusion criteria, all patients were considered “sensitive.” Upon entry into the study, all subjects (100%) had previously been diagnosed with contact dermtiatis and 9 (40.9%) subjects had active dermatitis with symptoms of dermatitis on their arms and/or hands. Subject demographic and baseline characteristics are presented in Table 11.2-1.

Table 11.2-1: Subject Demographics and Baseline Characteristics (All Enrolled Subjects—ITT Safety Population)

(page 1 of 2)

Number of Subjects	22
Age (years)	
N	22
Mean	49.6
STD	11.78
Median	52
Min. to Max.	28 to 65
Gender	
N	22
Male	5 (22.7%)
Female	17 (77.3%)
Ethnicity	
N	22
Scandinavian	21 (95.5%)
Bosnian	1 (4.6%)
Previous Positive Patch Test Results, Lyral[a]	
Weak (nonvesicular) positive reaction (+)	7 (31.8%)
Strong (vesicular) positive reaction (++)	15 (68.2%)
Extreme positive reaction (+++)	0 (0%)
Previous Positive Patch Test Results, Fragrance mix 2[a]	
Weak (nonvesicular) positive reaction (+)	5 (22.7%)
Strong (vesicular) positive reaction (++)	11 (50.0%)
Extreme positive reaction (+++)	2 (9.0%)
No previous reaction	4 (18.0%)

See SOURCE: Table 14.1.2 and Listing 16.2.1, Listing 16.2.2, Listing 16.2.9 p19, and Listing 16.2.9 p 20.

continued

Table 11.2-1: Subject Demographics and Baseline Characteristics: ITT Safety Population (page 2 of 2)

Type of Dermatitis[a]	
Allergic	3 (13.6%)
Irritant	3 (13.6%)
Atopic	3 (13.6%)
Occupational dermatitis	0 (0.0%)
Other[c]	2 (9.1%)
Subjects with Current Dermatitis Symptoms[a]	
Yes	9 (40.9%)
No	13 (59.0%)
Dermatitis Symptom Areas[b]	
Face and/or scalp and/or neck	1 (11.1%)
Trunk	1 (11.1%)
Arms and/or hands	8 (88.9%)
Legs and/or feet	3 (33.3%)

See SOURCE: Table 14.1.2 and Listing 16.2.1, Listing 16.2.2, Listing 16.2.9 p19, and Listing 16.2.9 p 20.

[a] Proportion based on number of subjects.

[b] Proportion based on number of subjects with dermatitis symptoms.

[c] Subject 104: lichen planus; Subject 110: unknown.

11.3 Measurements of Treatment Compliance

Subjects were randomized at Visit 1 to receive one of three differently configured T.R.U.E. TEST patches. All three configurations included the negative control and each of the test allergen concentrations. The T.R.U.E. TEST patches were applied to the back of every subject. After 2 days of occluded exposure, the patches were removed. No subject discontinued the study due to noncompliance, and no reports of noncompliance were recorded (Table 11.3-1).

Table 11.3-1 Treatment Compliance Summary Exposure-Time to Patch Panel: Safety Population

	Days of Exposure	Number of subjects
Investigational Panel	1	0
Investigational Panel	2	22
Chamber	1	0
Chamber	2	22

See SOURCE: Table 14.3.2.1

11.3.1 Assessment of Adhesion

Adhesion was assessed as excellent, good, poor, and detached. For both the ITT safety and PP populations, adhesion of both the test panels and the individual chamber with the reference allergen was determined to be excellent for all patients (100%, Table 11.3.1-1), and no factor appeared to have affected adhesion (Table 11.3.1-2). Furthermore, the condition of the skin was normal in the majority of patients in both the ITT safety and PP population (Table 11.3.1-3).

Table 11.3.1-1 Patch Panel Adhesion Scores: ITT Safety and PP Populations

	Investigational Test Panel								Chamber Panel							
	Excellent		Good		Poor		Detached		Excellent		Good		Poor		Detached	
Analysis Population	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	22	100.0	0	0.0	0	0.0	0	0.0	22	100.0	0	0.0	0	0.0	0	0.0
PP Population	20	100.0	0	0.0	0	0.0	0	0.0	20	100.0	0	0.0	0	0.0	0	0.0

ADHESION SCORE SCALE
Excellent : Skin contact good; all tape edges adherent; all allergens in contact with the skin
Good: Skin contact acceptable; some tape edges lifting; all allergens in contact with the skin
Poor : Little to no skin contact with panel; one or more allergens not in contact with the skin
Detached:Panel completely off the skin; none of the allergens in contact with the skin
See SOURCE: Table 14.2.10

Table 11.3.1-2 Summary of Factors that may have Affected Panel Adhesion: ITT Safety and PP Population

	None		Oily Skin		Excess hair		Wet panel		Sweat/skin moisture		Activity associated	
Analysis Population	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	22	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
PP Population	20	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.11

Table 11.3.1-3 Summary of Skin Condition: ITT Safety and PP Populations

	Normal		Dry		Oily	
Analysis Population	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	18	81.8	4	18.2	0	0.0
PP Population	17	85.0	3	15.0	0	0.0

See SOURCE: Table 14.2.12

11.4 Efficacy Results and Tabulations of Individual Subject Data

11.4.1 Analysis of Efficacy (Diagnostic Performance)

The primary endpoints included the optimal test allergen dose by determining the lowest concentration eliciting either +1 or +2 positive reactions in 70-90% of sensitive subjects; the frequency of positive, negative, doubtful, and irritant reactions for the reference allergen and for each test concentration of allergen; and the overall concordance and discordance between each test concentration and the reference allergen and between the test doses themselves.

11.4.1.1 Optimal Concentration

For the Lyral® T.R.U.E. TEST allergen, the optimal concentration was defined as the lowest concentration that elicited a positive skin reaction (+1 or +2) in 70% to 90% of the sensitive subjects (Table 11.4.1.1-1). Based on the investigator's determination of positive responses, the greatest proportion of subjects in the PP population who had a positive reaction to Lyral® on the T.R.U.E. TEST panel was 70% at the highest concentration of 0.40 mg/cm^2. Thus, this was the only concentration of the test allergen that elicited a positive response from at least 70% of the sensitive subjects to meet the criteria for the optimal dose. Based on the ITT safety population, 68% of the subjects had a positive reaction to this highest dose of Lyral®.

In contrast, 95% of the subjects in the PP population had a positive response to the reference allergen based on the investigator's determination. The percentage of positive responses in the

ITT safety population was also high (90.9%). Thus none of the test concentrations were as effective at eliciting positive responses as the reference allergen.

Table 11.4.1.1-1: Frequency of Positive Reactions by Investigator Determination

		Positive		Negative	
Analysis Population	**Allergen**	**n**	**%**	**n**	**%**
ITT (Safety) Population	Lyral® 0.40 mg/cm²	15	68.2	7	31.8
	Lyral® 0.20 mg/cm²	13	59.1	9	40.9
	Lyral® 0.10 mg/cm²	12	54.6	10	45.5
	Negative Control	-	-	22	100.0
	Chamber (Lyral® 5% in Petrolatum)	20	90.9	2	9.1
PP Population	Lyral® 0.40 mg/cm²	14	70.0	6	30.0
	Lyral® 0.20 mg/cm²	12	60.0	8	40.0
	Lyral® 0.10 mg/cm²	11	55.0	9	45.0
	Negative Control	-	-	20	100.0
	Chamber (Lyral® 5% in Petrolatum)	19	95.0	1	5.0

See SOURCE: Table 14.2.3 and Listing 16.2.5 and Listing 16.2.6

11.4.1.2 Reaction Frequencies

For each allergen concentration, the number and frequency of subjects with positive, negative, irritant, and doubtful reactions were tabulated for each visit. To identify general trends in the reaction frequencies, the outcomes for all enrolled subjects (ITT safety population) and for the PP population were reviewed separately at Visits 3, 4 and 5.

At Visit 3, at least 10 (45.5%) of the 22 enrolled subjects had positive reactions to the Lyral® T.R.U.E. TEST allergen at each concentration (range across doses, 10-15 subjects, 45.5%-68.2%). None of the 22 enrolled subjects had a positive reaction to the PVP control, and no subject had an irritant reaction to the PVP control or to any of the Lyral® T.R.U.E. TEST patch concentrations. In terms of the reference allergen (Lyral® in petrolatum), 16 patients (72.7%) had a positive reaction while 3 subjects (13.6%) had doubtful reactions; no patients had an irritant reaction (Table 11.4.1.2-1). Overall, the results in the PP population were similar to those of the ITT safety population.

At Visit 4, the proportions of all enrolled subjects with positive reactions to Lyral® (range across doses (9-14 subjects, 40.9%-63.6%) and the PVP control (0 subjects) were similar to those observed at Visit 3. Additionally, no subject had an irritant reaction to the PVP control, to any of the Lyral® T.R.U.E. TEST patch concentrations, or to the Lyral® in petrolatum reference allergen. Positive reactions to the reference allergen (17, 77.3%) were also similar to those at Visit 3 (Table 11.4.1.2-2).

At Visit 5, all reactions were negative for all doses of Lyral® and for the reference allergen in both the ITT safety and PP population (Table 11.4.1.2-3).

The positive skin reactions for Visits 3 and 4 are summarized in Table 11.4.1.2-4 and Table 11.4.1.2-5, respectively.

Table 11.4.1.2-1: Frequency of Positive, Negative, Irritant, and Doubtful Reactions at Visit 3 (Day 4) by Allergen and Concentration (ITT Safety (N = 22) and Per Protocol (N = 20) Populations)

Visit 3 Day 4		Neg		IR		?		1+		2+		3+	
Analysis Population	**Allergen**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	Lyral® 0.40 mg/cm²	3	13.6	0	0.0	4	18.2	5	22.7	10	45.5	0	0.0
	Lyral® 0.20 mg/cm²	6	27.3	0	0.0	4	18.2	6	27.3	6	27.3	0	0.0
	Lyral® 0.10 mg/cm²	9	40.9	0	0.0	3	13.6	7	31.8	3	13.6	0	0.0
	Negative Control	22	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chamber (Lyral® 5% in Petrolatum)	1	4.6	0	0.0	3	13.6	4	18.2	12	54.6	2	9.1
PP Population	Lyral® 0.40 mg/cm²	2	10.0	0	0.0	4	20.0	5	25.0	9	45.0	0	0.0
	Lyral® 0.20 mg/cm²	5	25.0	0	0.0	4	20.0	6	30.0	5	25.0	0	0.0
	Lyral® 0.10 mg/cm²	8	40.0	0	0.0	3	15.0	7	35.0	2	10.0	0	0.0
	Negative Control	20	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chamber (Lyral® 5% in Petrolatum)	0	0.0	0	0.0	3	15.0	4	20.0	11	55.0	2	10.0

See SOURCE: Table 14.2.2

KEY TO SCORING SKIN REACTIONS

Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Table 11.4.1.2-2: Frequency of Positive, Negative, Irritant, and Doubtful Reactions at Visit 4 (Day 7) by Allergen and Concentration (ITT Safety (N = 22) and Per Protocol (N = 20) Populations)

Visit 4 Day 7		Neg		IR		?		1+		2+		3+	
Analysis Population	**Allergen**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	Lyral® 0.40 mg/cm²	8	36.4	0	0.0	0	0.0	8	36.4	6	27.3	0	0.0
	Lyral® 0.20 mg/cm²	10	45.5	0	0.0	0	0.0	11	50.0	1	4.6	0	0.0
	Lyral® 0.10 mg/cm²	13	59.1	0	0.0	0	0.0	9	40.9	0	0.0	0	0.0
	Negative Control	22	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Chamber (Lyral®)5% in Petrolatum)	5	22.7	0	0.0	0	0.0	10	45.5	7	31.8	0	0.0
PP Population	Lyral® 0.40 mg/cm²	7	35.0	0	0.0	0	0.0	7	35.0	6	30.0	0	0.0
	Lyral® 0.20 mg/cm²	9	45.0	0	0.0	0	0.0	10	50.0	1	5.0	0	0.0
	Lyral® 0.10 mg/cm²	11	55.0	0	0.0	0	0.0	9	45.0	0	0.0	0	0.0
	Negative Control	20	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.2

KEY TO SCORING SKIN REACTIONS

Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Table 11.4.1.2-3: Frequency of Positive, Negative, Irritant, and Doubtful Reactions at Visit 5 (Day 21) by Allergen and Concentration (ITT Safety (N = 22) and Per Protocol (N = 20) Populations)

		Neg		1+		2+		3+	
Analysis Population	**Allergen**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	Lyral® 0.40 mg/cm²	22	100.0	0	0.00	0	0.00	0	0.00
	Lyral® 0.20 mg/cm²	22	100.0	0	0.00	0	0.00	0	0.00
	Lyral® 0.10 mg/cm²	22	100.0	0	0.00	0	0.00	0	0.00
	Negative Control	22	100.0	0	0.00	0	0.00	0	0.00
	Chamber (Lyral®)5% in Petrolatum)	22	100.0	0	0.00	0	0.00	0	0.00
PP Population	Lyral® 0.40 mg/cm²	20	100.0	0	0.00	0	0.00	0	0.00
	Lyral® 0.20 mg/cm²	20	100.0	0	0.00	0	0.00	0	0.00
	Lyral® 0.10 mg/cm²	20	100.0	0	0.00	0	0.00	0	0.00
	Negative Control	20	100.0	0	0.00	0	0.00	0	0.00
	Chamber (Lyral®) 5% in Petrolatum)	20	100.0	0	0.00	0	0.00	0	0.00

See SOURCE: **Table 14.2.2**

KEY TO SCORING SKIN REACTIONS

Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Table 11.4.1.2-4: Summary of Positive Skin Reactions for Visit 3 (Day 4) in the ITT Safety and PP Populations

Visit 3 Day 4			1+				2+				3+			
Analysis Population	**Allergen**	**Total N**	**n**	**%**	**95% Lower Confidence Limit**	**95% Upper Confidence Limit**	**n**	**%**	**95% Lower Confidence Limit**	**95% Upper Confidence Limit**	**n**	**%**	**95% Lower Confidence Limit**	**95% Upper Confidence Limit**
ITT (Safety) Population	Lyral® 0.40 mg/cm²	22	5	22.7	7.8	45.4	10	45.5	24.4	67.8	0	0.0	NC	NC
	Lyral® 0.20 mg/cm²	22	6	27.3	10.7	50.2	6	27.3	10.7	50.2	0	0.0	NC	NC
	Lyral® 0.10 mg/cm²	22	7	31.8	13.9	54.9	3	13.6	2.9	34.9	0	0.0	NC	NC
	Negative Control	22	0	0.0	NC	NC	0	0.0	NC	NC	0	0.0	NC	NC
	Chamber (Lyral® 5% in Petrolatum)	22	4	18.2	5.2	40.3	12	54.6	32.2	75.6	2	9.1	1.1	29.2
PP Population	Lyral® 0.40 mg/cm²	20	5	25.0	8.7	49.1	9	45.0	23.1	68.5	0	0.0	NC	NC
	Lyral® 0.20 mg/cm²	20	6	30.0	11.9	54.3	5	25.0	8.7	49.1	0	0.0	NC	NC
	Lyral® 0.10 mg/cm²	20	7	35.0	15.4	59.2	2	10.0	1.2	31.7	0	0.0	NC	NC
	Negative Control	20	0	0.0	NC	NC	0	0.0	NC	NC	0	0.0	NC	NC
	Chamber (Lyral® 5% in Petrolatum)	20	4	20.0	5.7	43.7	11	55.0	31.5	76.9	2	10.0	1.23	31.7

NC = Not calculated due to zero frequency

See SOURCE: Table 14.2.4

KEY TO SCORING SKIN REACTIONS
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Table 11.4.1.2-5: Summary of Positive Skin Reactions for Visit 4 (Day 7) in the ITT Safety and PP Populations

Visit 4 Day 7			1+				2+				3+			
Analysis Population	**Allergen**	**Total N**	**n**	**%**	**95% Lower Confidence Limit**	**95% Upper Confidence Limit**	**n**	**%**	**95% Lower Confidence Limit**	**95% Upper Confidence Limit**	**n**	**%**	**95% Lower Confidence Limit**	**95% Upper Confidence Limit**
ITT (Safety) Population	Lyral® 0.40 mg/cm²	22	8	36.4	17.2	59.3	6	27.3	10.7	50.2	0	0.0	NC	NC
	Lyral® 0.20 mg/cm²	22	11	50.0	28.2	71.8	1	4.6	0.1	22.8	0	0.0	NC	NC
	Lyral® 0.10 mg/cm²	22	9	40.9	20.7	63.7	0	0.0	NC	NC	0	0.0	NC	NC
	Negative Control	22	0	0.0	NC	NC	0	0.0	NC	NC	0	0.0	NC	NC
	Chamber (Lyral® 5% in Petrolatum)	22	10	45.5	24.4	67.8	7	31.8	13.9	54.9	0	0.0	NC	NC
PP Population	Lyral® 0.40 mg/cm²	20	7	35.0	15.4	59.2	6	30.0	11.9	54.3	0	0.0	NC	NC
	Lyral® 0.20 mg/cm²	20	10	50.0	27.2	72.8	1	5.0	0.1	24.9	0	0.0	NC	NC
	Lyral® 0.10 mg/cm²	20	9	45.0	23.1	68.5	0	0.0	NC	NC	0	0.0	NC	NC
	Negative Control	20	0	0.0	NC	NC	0	0.0	NC	NC	0	0.0	NC	NC
	Chamber (Lyral® 5% in Petrolatum)	20	10	50.0	27.2	72.8	6	30.0	11.9	54.3	0	0.0	NC	NC

See SOURCE: Table 14.2.4

NC = Not calculated due to zero frequency
KEY TO SCORING SKIN REACTIONS
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

11.4.1.3 Agreement of Skin Reactions and Concordance/Discordance

For both the ITT safety and PP populations, the percent agreement of skin reactions between the three T.R.U.E. TEST doses of Lyral® and the reference allergen (Lyral® 5% in petrolatum) were evaluated across Visits 3, 4, and 5 and severity of reaction.

At Visit 3 both for the ITT safety (Table 11.4.1.3-1) and the PP populations (Table 11.4.1.3-2), the highest percent agreement between reference allergen and T.R.U.E. TEST allergens was associated with the highest dose of the T.R.U.E. TEST allergen (0.40 mg/cm^2). The percent agreement in positive responses between the reference allergen and the T.R.U.E. TEST allergens decreased as the dose of the T.R.U.E. TEST allergen decreased while the percent agreement in terms of negative or doubtful responses was unchanged across doses. With the exception of a slight increase in the percent agreement (from 18.2% to 22.7%) between negative responses to the lowest dose of T.R.U.E. TEST allergen (0.10 mg/cm^2) and the reference allergen for the ITT and PP populations (from 15% to 20%), the same relationships were found at Visit 4. At Visit 5 all reactions were negative in both populations.

Table 11.4.1.3-1 Percent Agreement of Skin Reactions Across Doses (ITT Safety Population Visits 3, 4, and 5)

(page 1 of 3)

ITT (Safety) Population Visit 3 Day 4	Lyral® 0.40 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	1	4.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	1	4.6	0	0.0	1	4.6	1	4.6	0	0.0	0	0.0
1+	1	4.6	0	0.0	2	9.1	1	4.6	0	0.0	0	0.0
2+	0	0.0	0	0.0	1	4.6	3	13.6	8	36.4	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	2	9.1	0	0.0

See SOURCE: Table 14.2.5

ITT (Safety) Population Visit 3 Day 4	Lyral® 0.20 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	1	4.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	2	9.1	0	0.0	1	4.6	0	0.0	0	0.0	0	0.0
1+	3	13.6	0	0.0	1	4.6	0	0.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	2	9.1	6	27.3	4	18.2	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	2	9.1	0	0.0

See SOURCE: Table 14.2.5

ITT (Safety) Population Visit 3 Day 4	Lyral® 0.10 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	1	4.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	2	9.1	0	0.0	1	4.6	0	0.0	0	0.0	0	0.0
1+	4	18.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2+	2	9.1	0	0.0	2	9.1	6	27.3	2	9.1	0	0.0
3+	0	0.0	0	0.0	0	0.0	1	4.6	1	4.6	0	0.0

See SOURCE: Table 14.2.5

continued

Table 11.4.1.3-1 Percent Agreement of Skin Reactions Across Doses (ITT Safety Population Visits 3, 4, and 5)

(page 2 of 3)

ITT (Safety) Population Visit 4 Day 7	Lyral® 0.40 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	4	18.2	0	0.0	0	0.0	1	4.6	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	4	18.2	0	0.0	0	0.0	6	27.3	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	1	4.6	6	27.3	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

ITT (Safety) Population Visit 4 Day 7	Lyral® 0.20 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	4	18.2	0	0.0	0	0.0	1	4.6	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	6	27.3	0	0.0	0	0.0	4	18.2	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	6	27.3	1	4.6	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

ITT (Safety) Population Visit 4 Day 7	Lyral® 0.20 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	4	18.2	0	0.0	0	0.0	1	4.6	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	6	27.3	0	0.0	0	0.0	4	18.2	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	6	27.3	1	4.6	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

continued

Table 11.4.1.3-1 Percent Agreement of Skin Reactions Across Doses: ITT Safety Population Visits 3, 4, and 5

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ITT (Safety) Population Visit 5 Day 21	Lyral® 0.40 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	22	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

ITT (Safety) Population Visit 5 Day 21	Lyral® 0.20 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	22	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

ITT (Safety) Population Visit 5 Day 21	Lyral® 0.10 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	22	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

KEY TO SCORING SKIN REACTIONS

Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Table 11.4.1.3-2 Percent Agreement of Skin Reactions Across Doses: PP Population Visits 3, 4, and 5

(page 1 of 3)

PP Population Visit 3 Day 4	Lyral® 0.40 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	1	5.0	0	0.0	1	5.0	1	5.0	0	0.0	0	0.0
1+	1	5.0	0	0.0	2	10.0	1	5.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	1	5.0	3	15.0	7	35.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	2	10.0	0	0.0

See SOURCE: Table 14.2.5

PP Population Visit 3 Day 4	Lyral® 0.20 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	2	10.0	0	0.0	1	5.0	0	0.0	0	0.0	0	0.0
1+	3	15.0	0	0.0	1	5.0	0	0.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	2	10.0	6	30.0	3	15.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	2	10.0	0	0.0

See SOURCE: Table 14.2.5

PP Population Visit 3 Day 4	Lyral® 0.10 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	2	10.0	0	0.0	1	5.0	0	0.0	0	0.0	0	0.0
1+	4	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2+	2	10.0	0	0.0	2	10.0	6	30.0	1	5.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	1	5.0	1	5.0	0	0.0

See SOURCE: Table 14.2.5

continued

Table 11.4.1.3-2 Percent Agreement of Skin Reactions Across Doses: PP Population Visits 3, 4, and 5

(page 2 of 3)

PP Population Visit 4 Day 7	Lyral® 0.40 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	3	15.0	0	0.0	0	0.0	1	5.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	4	20.0	0	0.0	0	0.0	6	30.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	0	0.0	6	30.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

PP Population Visit 4 Day 7	Lyral® 0.20 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	3	15.0	0	0.0	0	0.0	1	5.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	6	30.0	0	0.0	0	0.0	4	20.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	5	25.0	1	5.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

PP Population Visit 4 Day 7	Lyral® 0.10 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	4	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	7	35.0	0	0.0	0	0.0	3	15.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	6	30.0	0	0.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

continued

Table 11.4.1.3-2 Percent Agreement of Skin Reactions Across Doses: PP Population Visits 3, 4, and 5

(page 3 of 3)

PP Population Visit 5 Day 21	Lyral® 0.40 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	20	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

PP Population Visit 5 Day 21	Lyral® 0.20 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	20	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

PP Population Visit 5 Day 21	Lyral® 0.10 mg/cm²											
	Neg		IR		?		1+		2+		3+	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	n	%	n	%	n	%	n	%
Neg	20	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
IR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
?	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3+	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

See SOURCE: Table 14.2.5

KEY TO SCORING SKIN REACTIONS

Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Concordance (or agreement) was based on positive reactions, which were defined as any positive reaction regardless of severity, and on nonnegative reactions, which were defined as negative, irritant, and doubtful reactions (Table 11.4.1.3-3). For both the ITT safety and PP populations, concordance/discordance, as measured by Cohen's Kappa, was evaluated across Visits 3 and 4 as follows:

- Between each dose of the T.R.U.E. TEST allergen and the associated reference allergen in petrolatum
- Between each dose of the T.R.U.E. TEST allergen and the other doses of the T.R.U.E. TEST allergen.

Because there were no reactions at Visit 5 in either population, Kappa was not calculated.

Kappa values > 0 and < 0.2 indicate poor concordance, values between 2 and < 0.4 indicate fair concordance, values between 0.4 and < 0.6 indicate moderate concordance, values between 0.6 and < 0.8 indicate good concordance, and values between 0.8 and 1.0 indicate very good concordance.

In the ITT safety population, concordance between all three doses of the T.R.U.E. TEST allergen and the reference allergen in petrolatum was moderate (Visit 3: Kappa = 4.86; Visit 4: Kappa = 0.4660) for the highest test dose. When the test dose was 0.20 mg/cm^2, concordance with the reference allergen was moderate at Visit 3 (Kappa = 0.4211) and fair at Visit 4 (Kappa = 0.3304). Concordance between the lowest allergen test dose of 0.10 mg/cm^2 and the reference allergen was fair at both Visit 3 (Kappa = 0.3125) and Visit 4 (Kappa = 0.3383). Hence, concordance with the reference allergen tended to decrease as the test dose decreased (Table 11.4.1.3-3).

In the PP population, concordance between the three test doses of the T.R.U.E. TEST allergen and the reference allergen in petrolatum was fair (Table 11.4.1.3-4) and tended to be slightly lower than that in the ITT safety population. Concordance between the highest dose (0.40 mg/cm^2) and the reference allergen was moderate and increased from Visit 3 (Kappa = 0.3056) to Visit 4 (Kappa = 0.3902). For the middle test dose of 0.20 mg/cm^2, concordance decreased from Visit 3 (Kappa = 0.3548) to Visit 4 (Kappa = 0.2553). Concordance between the lowest test dose (0.10 mg/cm^2) and the reference allergen increased from Visit 3 (Kappa = 0.2523) to Visit 4 (Kappa = 0.3396).

Table 11.4.1.3-3 Concordance Between Reference Allergen and Test Doses: ITT Safety Population at Visits 3 and 4

(page 1 of 2)

ITT (Safety) Population	Lyral® 0.40 mg/cm²				
	Not Positive		Positive		
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	3	13.6	1	4.6	**0.4086**
Positive	4	18.2	14	63.6	
Visit 4 Day 7					
Not Positive	4	18.2	1	4.6	**0.4660**
Positive	4	18.2	13	59.1	
Visit 5 Day 21					
Not Positive	22	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6a and Table 14.2.6.b

ITT (Safety) Population	Lyral® 0.20 mg/cm²				
	Not Positive		Positive		
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	4	18.2	0	0.0	**0.4211**
Positive	6	27.3	12	54.6	
Visit 4 Day 7					
Not Positive	4	18.2	1	4.6	**0.3304**
Positive	6	27.3	11	50.0	
Visit 5 Day 21					
Not Positive	22	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6c and Table 14.2.6d

Table 11.4.1.3-3 Concordance Between Reference Allergen and Test Doses: ITT Safety Population at Visits 3 and 4

(page 2 of 2)

ITT (Safety) Population	Lyral® 0.10 mg/cm²				
	Not Positive		Positive		
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	4	18.2	0	0.0	**0.3125**
Positive	8	36.4	10	45.5	
Visit 4 Day 7					
Not Positive	5	22.7	0	0.0	**0.3383**
Positive	8	36.4	9	41.0	
Visit 5 Day 21					
Not Positive	22	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6e and Table 14.2.6e
NC = Not calculated due to zero frequency
KEY : Not Positive includes (Neg, IR and ?), Positive includes 1+, 2+, 3+
Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Table 11.4.1.3-4 Concordance Between Reference Allergen and Test Doses: PP Population at Visits 3 and 4

(page 1 of 2)

PP Population	Lyral® 0.40 mg/cm²				
	Not Positive		Positive		
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	2	10.0	1	5.0	**0.3056**
Positive	4	20.0	13	65.0	
Visit 4 Day 7					
Not Positive	3	15.0	1	5.0	**0.3902**
Positive	4	20.0	12	60.0	
Visit 5 Day 21					
Not Positive	20	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6a and Table 14.2.6b

PP Population	Lyral® 0.20 mg/cm²				
	Not Positive		Positive		
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	3	15.0	0	0.0	**0.3548**
Positive	6	30.0	11	55.0	
Visit 4 Day 7					
Not Positive	3	15.0	1	5.0	**0.2553**
Positive	6	30.0	10	50.0	
Visit 5 Day 21					
Not Positive	20	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE; Table 14.2.6c and Table 14.2.6d

continued

Table 11.4.1.3-4 Concordance Between Reference Allergen and Test Doses: PP Population at Visits 3 and 4

(page 2 of 2)

PP Population	Lyral® 0.10 mg/cm²				
	Not Positive		Positive		
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	3	15.0	0	0.0	**0.2523**
Positive	8	40.0	9	45.0	
Visit 4 Day 7					
Not Positive	4	20.0	0	0.0	**0.3396**
Positive	7	35.0	9	45.0	
Visit 5 Day 21					
Not Positive	20	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6e and Table 14.2.6f
NC = Not calculated due to zero frequency
KEY : Not Positive includes (Neg, IR and ?), Positive Includes 1+, 2+, 3+)
Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

For both the ITT safety and PP populations, concordance calculated among the allergen test doses (Table 11.4.1.3-5 and Table 11.4.1.3-6) at Days 3 and 4 ranged from moderate to very good.

Table 11.4.1.3-5 Concordance Among Test Doses: ITT Safety Population at Visits 3 and 4

(page 1 of 1)

ITT (Safety) Population	Lyral® 0.40 mg/cm²				
	Not Positive		Positive		
Lyral® 0.20 mg/cm²	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	7	31.8	3	13.6	**0.7179**
Positive	0	0.0	12	54.6	
Visit 4 Day 7					
Not Positive	8	36.4	2	9.1	**0.8136**
Positive	0	0.0	12	54.6	
Visit 5 Day 21					
Not Positive	22	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6a and Table 14.2.6b

ITT (Safety) Population	Lyral® 0.40 mg/cm²				
	Not Positive		Positive		
Lyral® 0.10 mg/cm²	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	7	31.8	5	22.7	**0.5600**
Positive	0	0.0	10	45.5	
Visit 4 Day 7					
Not Positive	8	36.4	5	22.7	**0.5669**
Positive	0	0.0	9	40.9	
Visit 5 Day 21					
Not Positive	22	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6.c and Table 14.2.6d

ITT (Safety) Population	Lyral® 0.20 mg/cm²				
	Not Positive		Positive		
Lyral® 0.10 mg/cm²	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	10	45.5	2	9.1	**0.8197**
Positive	0	0.0	10	45.5	
Visit 4 Day 7					
Not Positive	10	45.5	3	13.6	**0.7317**
Positive	0	0.0	9	40.9	
Visit 5 Day 21					
Not Positive	22	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6e and Table 14.2.6f
NC = Not calculated due to zero frequency
KEY : Not Positive includes (Neg, IR and ?), Positive Includes 1+, 2+, 3+
Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Table 11.4.1.3-6 Concordance Among Test Doses: PP Population at Visits 3 and 4

(page 1 of 1)

PP Population	Lyral® 0.40 mg/cm²				
	Not Positive		Positive		
Lyral® 0.20 mg/cm²	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	6	30.0	3	15.0	**0.6875**
Positive	0	0.0	11	55.0	
Visit 4 Day 7					
Not Positive	7	35.0	2	10.0	**0.7938**
Positive	0	0.0	11	55.0	
Visit 5 Day 21					
Not Positive	20	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6a and Table 14.2.6b

PP Population	Lyral® 0.40 mg/cm²				
	Not Positive		Positive		
Lyral® 0.10 mg/cm²	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	6	30.0	5	25.0	**0.5192**
Positive	0	0.0	9	45.0	
Visit 4 Day 7					
Not Positive	7	35.0	4	20.0	**0.6117**
Positive	0	0.0	9	45.0	
Visit 5 Day 21					
Not Positive	20	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6c and Table 14.2.6d

PP Population	Lyral® 0.20 mg/cm²				
	Not Positive		Positive		
Lyral® 0.10 mg/cm²	n	%	n	%	Cohen's Kappa
Visit 3 Day 4					
Not Positive	9	45.0	2	10.0	**0.8020**
Positive	0	0.0	9	45.0	
Visit 4 Day 7					
Not Positive	9	45.0	2	10.0	**0.8020**
Positive	0	0.0	9	45.0	
Visit 5 Day 21					
Not Positive	20	100.0	0	0.0	NC
Positive	0	0.0	0	0.0	

See SOURCE: Table 14.2.6e and Table 14.2.6f
NC = Not calculated due to zero frequency
KEY : Not Positive includes (Neg, IR and ?), Positive Includes 1+, 2+, 3+)
Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+: Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Based on the investigator's determination of positive reactions (Table 11.4.1.3-7 and Table 11.4.1.3-8), the greatest observed concordance between the test allergens and the reference allergen occurred at the test concentration of 0.40 mg/cm^2 both in the ITT population (Kappa = 0.3529, % concordance = 77.3%, confidence interval (CI) = 58.0-92.5) and in the PP population (Kappa = 0.2187, % concordance = 75%, CI = 60.4-94.0). Therefore, the highest level of concordance attained between the test doses and the reference allergen was fair. Furthermore, concordance in both populations decreased as the dose decreased (Table 11.4.1.3-7 and Table 11.4.1.3-8).

Table 11.4.1.3-7 Concordance/Discordance Between Test Allergens and Reference Allergen (Investigator Determination of Positive Reactions): ITT Population

(page 1 of 1)

ITT (Safety) Population	Lyral® 0.40 mg/cm²			
	Not Positive		Positive	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%
Not Positive	2	9.1	0	0.0
Positive	5	22.7	15	68.2

	n	%	95% CI	Cohen's Kappa
Concordance	17	77.3	58.0– 92.5	**0.3529**
Discordance	5	22.7	7.5 – 42.0	

See SOURCE: Table 14.2.7 and Table 14.2.8

ITT (Safety) Population	Lyral® 0.20 mg/cm²			
	Not Positive		Positive	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%
Not Positive	2	9.1	0	0.0
Positive	7	31.8	13	59.1

	n	%	95% CI	Cohen's Kappa
Concordance	15	68.2	53.6 – 89.1	**0.2524**
Discordance	7	31.8	10.9 – 46.4	

See SOURCE: Table 14.2.7 and Table 14.2.8

ITT (Safety) Population	Lyral® 0.10 mg/cm²			
	Not Positive		Positive	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%
Not Positive	2	9.1	0	0.0
Positive	8	36.4	12	54.6

	n	%	95% CI	Cohen's Kappa
Concordance	14	63.6	51.5 – 87.3	**0.2143**
Discordance	8	36.4	12.8 – 48.5	

See SOURCE: Table 14.2.7 and Table 14.2.8

KEY : Not Positive includes (Neg, IR and ?), Positive Includes 1+, 2+, 3+)
Neg Negative reaction: No Reaction
IR Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
? Doubtful: Faint macular or homogenous erythema with no infiltration
1+ Weak Positive: Erythema, Infiltration, Discrete papules
2+ Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+ Extreme Positive: Coalescing vesicles, Bullous reaction.

Table 11.4.1.3-8 Concordance/Discordance Between Test Allergens and Reference Allergen (Investigator Determination): PP Population

(page 1 of 1)

PP Population	Lyral® 0.40 mg/cm²			
	Not Positive		Positive	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%
Not Positive	1	5.0	0	0.0
Positive	5	25.0	14	70.0

	n	%	95% CI	Cohen's Kappa
Concordance	15	75.0	60.4 – 94.0	**0.2187**
Discordance	5	25.0	6.0 – 39.6	

See SOURCE: Table 14.2.7 and Table 14.2.8

PP Population	Lyral® 0.20 mg/cm²			
	Not Positive		Positive	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%
Not Positive	1	5.0	0	0.0
Positive	7	35.0	12	60.0

	n	%	95% CI	Cohen's Kappa
Concordance	13	65.0	55.6 – 90.4	**0.1463**
Discordance	7	35.0	9.6 – 44.4	

See SOURCE: Table 14.2.7 and Table 14.2.8

PP Population	Lyral® 0.10 mg/cm²			
	Not Positive		Positive	
Chamber (Lyral® 5% in Petrolatum)	n	%	n	%
Not Positive	1	5.0	0	0.0
Positive	8	40.0	11	55.0

	n	%	95% CI	Cohen's Kappa
Concordance	12	60.0	53.3 – 88.4	**0.1209**
Discordance	8	40.0	11.6 – 46.7	

See SOURCE: Table 14.2.7 and Table 14.2.8

KEY : Not Positive includes (Neg, IR and ?), Positive Includes 1+, 2+, 3+)

Neg Negative reaction: No Reaction
IR Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
? Doubtful: Faint macular or homogenous erythema with no infiltration
1+ Weak Positive: Erythema, Infiltration, Discrete papules
2+ Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+ Extreme Positive: Coalescing vesicles, Bullous reaction.

Based on the investigator's determination of positive reactions, concordance was also calculated among the test doses of allergen both for the ITT safety population (Table 11.4.1.3-9) and the PP population (Table 11.4.1.3-10). In both populations concordance among the doses ranged from good to very good.

Overall, however, the concordances observed between test and reference allergens indicate that the three doses of the T.R.U.E. TEST allergen were not as efficacious in eliciting positive reactions as the reference allergen in petrolatum. The high concordance among the test doses of allergens indicates that the different doses were associated with similar results.

Table 11.4.1.3-9 Concordance/Discordance Among Test Allergens (Investigator Determination): ITT Population

(page 1 of 1)

ITT (Safety) Population	Lyral® 0.40 mg/cm²			
	Not Positive		Positive	
Lyral® 0.20 mg/cm²	n	%	n	%
Not Positive	7	31.8	2	9.1
Positive	0	0.0	13	59.1

	n	%	95% CI	Cohen's Kappa
Concordance	20	90.9	78.9 – 102.9	**0.8053**
Discordance	2	9.1	-2.9 – 21.1	

See SOURCE: Table 14.27 and Table 14.2.8

ITT (Safety) Population	Lyral® 0.40 mg/cm²			
	Not Positive		Positive	
Lyral® 0.10 mg/cm²	n	%	n	%
Not Positive	7	31.8	3	13.6
Positive	0	0.0	12	54.6

	n	%	95% CI	Cohen's Kappa
Concordance	19	86.4	72.0 – 100.7	**0.7179**
Discordance	3	13.6	-0.7 – 28.0	

See SOURCE: Table 14.27 and Table 14.2.8

ITT (Safety) Population	Lyral® 0.20 mg/cm²			
	Not Positive		Positive	
Lyral® 0.10 mg/cm²	n	%	n	%
Not Positive	9	40.9	1	4.6
Positive	0	0.0	12	54.6

	n	%	95% CI	Cohen's Kappa
Concordance	21	95.5	86.8 – 104.2	**0.9076**
Discordance	1	4.6	-4.2 – 13.3	

See SOURCE: Table 14.2.7 and Table 14.2.8

KEY : Not Positive includes (Neg, IR and ?), Positive Includes 1+, 2+, 3+)

Neg Negative reaction: No Reaction
IR Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
? Doubtful: Faint macular or homogenous erythema with no infiltration
1+ Weak Positive: Erythema, Infiltration, Discrete papules
2+ Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+ Extreme Positive: Coalescing vesicles, Bullous reaction.

Table 11.4.1.3-10 Concordance/Discordance Among Test Allergens (Investigator Determination): PP Population

(page 1 of 1)

PP Population	Lyral® 0.40 mg/cm²			
	Not Positive		Positive	
Lyral® 0.20 mg/cm²	n	%	n	%
Not Positive	6	30.0	2	10.0
Positive	0	0.0	12	60.0

	n	%	95% CI	Cohen's Kappa
Concordance	18	90.0	76.9 – 103.2	**0.7826**
Discordance	2	10.0	-3.2 – 23.2	

See SOURCE: Table 14.2.7 and Table 14.2.8

PP Population	Lyral® 0.40 mg/cm²			
	Not Positive		Positive	
Lyral® 0.10 mg/cm²	n	%	n	%
Not Positive	6	30.0	3	15.0
Positive	0	0.0	11	55.0

	n	%	95% CI	Cohen's Kappa
Concordance	17	85.0	69.4 – 100.7	**0.6875**
Discordance	3	15.0	-0.7 – 30.7	

See SOURCE: Table 14.2.7 and Table 14.2.8

PP Population	Lyral® 0.20 mg/cm²			
	Not Positive		Positive	
Lyral® 0.10 mg/cm²	n	%	n	%
Not Positive	8	40.0	1	5.0
Positive	0	0.0	11	55.0

	n	%	95% CI	Cohen's Kappa
Concordance	19	95.0	85.5 – 104.6	**0.8980**
Discordance	1	5.0	-4.6 – 14.6	

See SOURCE: Table 14.2.7 and Table 14.2.8

KEY : Not Positive includes (Neg, IR and ?), Positive Includes 1+, 2+, 3+)

Neg Negative reaction: No Reaction
IR Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
? Doubtful: Faint macular or homogenous erythema with no infiltration
1+ Weak Positive: Erythema, Infiltration, Discrete papules
2+ Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+ Extreme Positive: Coalescing vesicles, Bullous reaction.

11.4.1.4 Generalized Estimation Equations Analysis

The frequency of ranked skin responses (positive 1+, 2+, 3+, negatives, and irritant reactions) was calculated for each allergen test dose. Statistical differences among the dose-related frequencies were evaluated within each allergen for Visits 3 and 4 for both the ITT safety and PP populations using the Generalized Estimation Equations (GEE) approach (Table 11.4.1.4-1 and Table 11.4.1.4-2). All statistical significance was based on two-sided hypothesis testing at the p = 0.05 level.

In both the ITT safety and PP populations, all three doses of the T.R.U.E. TEST allergen were significantly different than the negative control for both Visits 3 and 4 (Table 11.4.1.4-1 and Table 11.4.1.4-2). The overall dose response p values from the GEE analysis were also significant for the ITT safety population (p = 0.0004) and PP population (p = 0.0006) for Visit 3 and again for Visit 4 (ITT safety population, p = 0.0004; PP population, p = 0.0082). However, at Visit 3, the only significant difference (p = 0.0103) among the test doses was between the highest (0.40 mg/cm^2) and lowest (0.10 mg/cm^2) doses in the ITT safety population; no significant differences were found for the PP population at that visit (Table 11.4.1-4-1). At Visit 4, the same significant difference was found in the ITT safety population, but significant differences were also found in the PP population between the highest (0.40 mg/cm^2) and middle (0.20 mg/cm^2) doses (p = 0.0081) and between the highest (0.40 mg/cm^2) and lowest (0.10 mg/cm^2) doses (p = 0.0015).

Based on the investigator's determination of positive reactions (Table 11.4.1.4-3), the overall dose response p value from the GEE analysis was significant (p = 0.0018) for the ITT safety population and for the PP population (p = 0.0029). The results of the multiple comparison testing indicated that all three test allergen doses in both populations were significantly different than the negative control (Table 11.4.1.4-3), but there no significant differences among any of the doses of the test allergens.

Table 11.4.1.4-1: GEE Analysis: ITT and PP Populations Visit 3

(page 1 of 1)

Visit 3 Day 4		Neg		IR		?		1+		2+		3+	
Analysis Population	**Allergen**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	Lyral® 0.40 mg/cm²	3	13.6	0	0.0	4	18.2	5	22.7	10	45.5	0	0.0
	Lyral® 0.20 mg/cm²	6	27.3	0	0.0	4	18.2	6	27.3	6	27.3	0	0.0
	Lyral® 0.10 mg/cm²	9	40.9	0	0.0	3	13.6	7	31.8	3	13.6	0	0.0
	Negative Control	22	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Overall Dose Response p-value from GEE analysis: **0.0004**[1] Significant Comparisons: Lyral® 0.40, Lyral® 0.20, Lyral® 0.10 vs. Negative Control List comparisons and p-values: Lyral® 0.40 vs. Negative Control:**<0.0001**[1] Lyral® 0.20 vs. Negative Control:**0.0001**[1] Lyral® 0.10 vs. Negative Control:**0.0007**[1] Lyral® 0.40 vs. Lyral® 0.20:0.0801 Lyral® 0.40 vs. Lyral® 0.10:**0.0103**[1] Lyral® 0.20 vs. Lyral® 0.10:0.2254													
PP Population	Lyral® 0.40 mg/cm²	2	10.0	0	0.0	4	20.0	5	25.0	9	45.0	0	0.0
	Lyral® 0.20 mg/cm²	5	25.0	0	0.0	4	20.0	6	30.0	5	25.0	0	0.0
	Lyral® 0.10 mg/cm²	8	40.0	0	0.0	3	15.0	7	35.0	2	10.0	0	0.0
	Negative Control	20	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Overall Dose Response p-value from GEE analysis: **0.0006**[1] Significant Comparisons: Lyral® 0.40, Lyral® 0.20, Lyral® 0.10 vs. Negative Control List comparisons and p-values: Lyral® 0.40 vs. Negative Control:**0.0001**[1] Lyral® 0.20 vs. Negative Control:**0.0007**[1] Lyral® 0.10 vs. Negative Control:**0.0037**[1] Lyral® 0.40 vs. Lyral® 0.20:0.3269 Lyral® 0.40 vs. Lyral® 0.10:0.1166 Lyral® 0.20 vs. Lyral® 0.10:0.3705													

See SOURCE: Table 14.2.2 and Table 14.2.9

KEY TO SCORING SKIN REACTIONS
Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+; Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction
[1] Significance difference

Table 11.4.1.4-2: GEE Analysis: ITT and PP Populations Visit 4

(page 1 of 1)

Visit 4 Day 7		Neg		IR		?		1+		2+		3+	
Analysis Population	**Allergen**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	Lyral® 0.40 mg/cm²	8	36.4	0	0.0	0	0.0	8	36.4	6	27.3	0	0.0
	Lyral® 0.20 mg/cm²	10	45.5	0	0.0	0	0.0	11	50.0	1	4.6	0	0.0
	Lyral® 0.10 mg/cm²	13	59.1	0	0.0	0	0.0	9	40.9	0	0.0	0	0.0
	Negative Control	22	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Overall Dose Response p-value from GEE analysis:**0.0004**[1] Significant Comparisons: Lyral® 0.40, Lyral® 0.20, Lyral® 0.10 vs. Negative Control List comparisons and p-values: Lyral® 0.40 vs. Negative Control:**<0.0001**[1] Lyral® 0.20 vs. Negative Control:**0.0001**[1] Lyral® 0.10 vs. Negative Control:**0.0007**[1] Lyral® 0.40 vs. Lyral® 0.20:0.0801 Lyral® 0.40 vs. Lyral® 0.10:**0.0103**[1] Lyral® 0.20 vs. Lyral® 0.10:0.2254													
PP Population	Lyral® 0.40 mg/cm²	7	35.0	0	0.0	0	0.0	7	35.0	6	30.0	0	0.0
	Lyral® 0.20 mg/cm²	9	45.0	0	0.0	0	0.0	10	50.0	1	5.0	0	0.0
	Lyral® 0.10 mg/cm²	11	55.0	0	0.0	0	0.0	9	45.0	0	0.0	0	0.0
	Negative Control	20	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Overall Dose Response p-value from GEE analysis: **0.0082**[1] Significant Comparisons: Lyral® 0.40, Lyral® 0.20, Lyral® 0.10 vs. Negative Control List comparisons and p-values: Lyral® 0.40 vs. Negative Control:**0.0006**[1] Lyral® 0.20 vs. Negative Control:**0.0013**[1] Lyral® 0.10 vs. Negative Control:**0.0026**[1] Lyral® 0.40 vs. Lyral® 0.20:**0.0081**[1] Lyral® 0.40 vs. Lyral® 0.10:**0.0015**[1] Lyral® 0.20 vs. Lyral® 0.10:0.0832													

See SOURCE: Table 14.2.2 and Table 14.2.9

[1] Significance difference

KEY TO SCORING SKIN REACTIONS

Neg: Negative reaction: No Reaction
IR: Irritant reaction; discrete, patchy, follicular, or homogenous erythema with no infiltration
?: Doubtful: Faint macular or homogenous erythema with no infiltration
1+: Weak Positive: Erythema, Infiltration, Discrete papules
2+; Strong Positive: Erythema, Papules, Infiltration, Discrete vesicles
3+: Extreme Positive: Coalescing vesicles, Bullous reaction

Table 11.4.1.4-3: GEE Analysis: ITT and PP Populations Based on Investigator's Determination of Positive Reactions

(page 1 of 1)

		Positive		Negative	
Analysis Population	**Allergen**	**n**	**%**	**n**	**%**
ITT (Safety) Population	Lyral® 0.40 mg/cm²	15	68.2	7	31.8
	Lyral® 0.20 mg/cm²	13	59.1	9	40.9
	Lyral® 0.10 mg/cm²	12	54.6	10	45.5
	Negative Control	.	.	22	100.0
Overall Dose Response p-value from GEE analysis: **0.0018**[1] Significant Comparisons: Lyral® 0.40, Lyral® 0.20, Lyral® 0.10 vs. Negative Control List comparisons and p-values: Lyral® 0.40 vs. Negative Control:**0.0001**[1] Lyral® 0.20 vs. Negative Control:**0.0003**[1] Lyral® 0.10 vs. Negative Control:**0.0005**[1] Lyral® 0.40 vs. Lyral® 0.20:0.1572 Lyral® 0.40 vs. Lyral® 0.10:0.0832 Lyral® 0.20 vs. Lyral® 0.10:0.3173					
PP Population	Lyral® 0.40 mg/cm²	14	70.0	6	30.0
	Lyral® 0.20 mg/cm²	12	60.0	8	40.0
	Lyral® 0.10 mg/cm²	11	55.0	9	45.0
	Negative Control	.	.	20	100.0
Overall Dose Response p-value from GEE analysis:**0.0029**[1] Significant Comparisons: Lyral® 0.40, Lyral® 0.20, Lyral® 0.10 vs. Negative Control List comparisons and p-values: Lyral® 0.40 vs. Negative Control:**0.0001**[1] Lyral® 0.20 vs. Negative Control:**0.0005**[1] Lyral® 0.10 vs. Negative Control:**0.0009**[1] Lyral® 0.40 vs. Lyral® 0.20:0.1572 Lyral® 0.40 vs. Lyral® 0.10:0.0832 Lyral® 0.20 vs. Lyral® 0.10:0.3173					

See SOURCE: Table 14.2.2 and Table 14.2.9

[1] Significant difference

11.4.2 Statistical/Analytical Issues

11.4.2.1 Adjustments for Covariates

No adjustments were made for covariates.

11.4.2.2 Handling of Dropouts or Missing Data

No imputations for missing data were performed.

11.4.2.3 Interim Analyses and Data Monitoring

There were no interim analyses and no data monitoring was performed in this study.

11.4.2.4 Multicenter Studies

Not applicable. This was a single-center study.

11.4.2.5 Multiple Comparisons/Multiplicity

No adjustments were made for multiple comparisons.

11.4.2.6 Use of an "Efficacy Subset" of Subjects?

Where applicable, two analysis subsets were evaluated. The intent-to-treat (ITT) safety population was defined as all enrolled subjects (n = 22) who received a patch application. This population was used to analyze safety and to support primary endpoints. The per-protocol population included all subjects who received a patch application and who completed the study with no major protocol violations (n = 20). This population was used to evaluate the the primary study endpoints. Results for these populations, along with comparisons of the results across populations where applicable, are presented within Section 11.4.1.

11.4.2.7 Active-Control Studies Intended to Show Equivalence

This was not an active-controlled study intended to show equivalence. Rather, the objective of this study was to evaluate the diagnostic performance and safety of three concentrations of Lyral® T.R.U.E. TEST allergen.

11.4.3 Tabulation of Individual Response Data

Individual efficacy response data are appended (see Appendix 16.2.6).

11.4.4 Drug Dose, Drug Concentration, and Relationships to Response

This was a concentration-response study in which three concentrations of Lyral® were evaluated for their diagnostic performance within T.R.U.E. TEST patches. No active pharmaceutical product was included in this study; thus, evaluations of drug dose, drug concentration, and relationship to response are not applicable. Results for the diagnostic performance of each allergen concentration are presented within Section 11.4.1.

11.4.5 Drug-Drug and Drug-Disease Interactions

No active pharmaceutical products were evaluated in this study, and thus no drug-drug or drug-disease interactions were evaluated.

11.4.6 By-Subject Displays

No formal, by-subject displays were produced. However, information in each of the listings in Appendix 16.2 is presented by subject.

11.4.7 Efficacy Conclusions

This study evaluated the diagnostic performance of three concentrations of Lyral® within T.R.U.E. TEST patches. A summary of the efficacy (i.e., performance) results follows.

- Based on the investigator's determination of positive responses, the greatest proportion of subjects in the PP population who had a positive reaction to Lyral® on the T.R.U.E. TEST panel was 70% at the highest concentration of 0.40 mg/cm^2. Thus, this was the only concentration of the test allergen that elicited a positive response at least 70% of the sensitive subjects to meet the criteria for the optimal dose.

- In contrast, the reference allergen of Lyral® 5% in petrolatum elicited 91% positive reactions (20 of 22 responses were positive in the ITT safety population). Hence, the reference allergen was more efficacious than the T.R.U.E. TEST doses of allergen in eliciting positive reactions.

- For the ITT safety population across visits, the Cohen's Kappa values ranged from 0.313 to 0.466, indicating fair to moderate agreement between the Lyral® doses and the reference allergen in petrolatum. Based on the investigator's determination of positive reactions, the agreement between the T.R.U.E. TEST doses of allergen and the reference allergen was also fair to moderate.

- When the the Lyral® doses were compared to each other across visits, the values of Kappa ranged from 0.567 -0.820 indicating moderate to very good agreement. In other words, the different doses were associated with similar results. When the Lyral® doses were compared to each other in terms of the investigator's determination of positive reactions, the

concordance increased, ranging from 0.718 to 0.908 and indicating substantial agreement among the doses.

- For the ITT safety population at Visits 3 and 4, the GEE analysis indicated a significant overall difference between treatments. The results of the multiple comparison analysis indicated a difference between the Lyral® doses 0.40, 0.20, and 0.10 mg/cm^2 and the negative control. Additionally, the Lyral® 0.40 dose was statistically different than the Lyral®0.10 dose.
- Based on the investigator's determination of positive reactions, the GEE analysis indicated a significant overall difference between treatments. The results of the multiple comparisons analysis indicated a difference between the Lyral® doses (0.40, 0.20, and 0.10 mg/cm^2) and the negative control.

12. SAFETY EVALUATION

12.1 Extent of Exposure

Subjects were randomized at Visit 1 to receive one of three differently configured T.R.U.E. TEST patches. All three configurations included the negative control and each of the test allergen concentrations (i.e. 0.0, 0.10, 0.20, and 0.40 mg/cm^2 Lyral®). The T.R.U.E. TEST patches were applied to the back of every subject as was a single chamber with a reference allergen (Lyral® 5% in petrolatum). After 2 days of occluded exposure, the patches and the chamber were removed. Thus, the extent of exposure was the same for all enrolled subjects.

12.2 Adverse Events

In this study, an AE was defined as any untoward medical occurrence in a study subject who applied the T.R.U.E. TEST patch. The event did not necessarily have to have a causal relationship with the patch or its usage. Reporting of all AEs began following patch application at Visit 1 and ended at the last study visit (Visit 5). Any known AEs that were considered possibly related to the study that occurred following Visit 5 also were reported.

12.2.1 Brief Summary of Adverse Events

Of the 22 subjects enrolled in the study who were exposed to the study product, three subjects (13.6%) reported one AE each (Table 12.2.1-1 and Table 12.2.1-2). All three events were deemed mild and nonserious. The first event occurred in a female and was reported as conjunctivitis; the event was not considered related to the study panels. The patient was treated with Levocabastine/Livostine Eye Drops, and the conjunctivitis resolved. The second (in a male) and third (in a female) events, which were both reported as “pruritus at the application site, were both treated with topical Ungv. Dermovate and both resolved. Only the second event in the male was considered “possibly related” to the study panel. Overall, the use of the study product resulted in no safety signals.

Table 12.2.1-1 Overview of Adverse Events: ITT Safety Population

N=22 (total number of subjects)[1]	Number of Subjects (%)	
Number of subjects experiencing an adverse event	3	13.6
Number of subjects experiencing a serious adverse event	0	0.0
Serious		
Non Serious (No. of subjects)	0	0.0
Serious (No. of subjects)	3	100.0
Severity of adverse events		
Mild (No. of subjects)	3	100.0
Moderate (No. of subjects)	0	0.0
Severe (No. of subjects)	0	0.0
Relationship of adverse events to treatment		
Definitely Related (No. of subjects)	0	0.0
Possibly Related (No. of subjects)	1	33.3
Not Related (No. of subjects)	2	66.7

[1]Subjects may fall into more than 1 category. See SOURCE: Table 14.3.1.4

Table 12.2.1-2 Adverse Events: Number Observed and Rate

N=22 Total AEs	Mild		Moderate		Severe		Total		Total
	Related N (%)	NR N (%)	Related N (%)	NR N (%)	Related N (%)	NR N (%)	Related N (%)	NR N (%)	R+NR N (%)
Total Adverse Events	1 (33.3%)	2 (66.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	2 (66.7%)	3 (100.0%)
Body System									
Eye Disorders	0 (0.0%)	1 (4.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.6%)	1 (4.6%)
General Disorders And Administration Site Conditions	1 (4.6%)	1 (4.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (4.6%)	1 (4.6%)	2 (9.1%)

NOTE: Related adverse event was categorized as possibly. See SOURCE: Table 14.3.1.1, Table 14.3.1.2 and Table 14.3.1.3

12.2.2 Display of Adverse Events

The three events reported in the study were described as “conjunctivitis” and “pruritis at the application site.” A summary of AE frequency is presented in Table 12.2.1-1 and Table 12.2.1-2.

12.2.3 Analysis of Adverse Events

Of the 22 enrolled subjects, 3 reported AEs. Neither AE was serious or severe, and neither resulted in subject discontinuation from the study. Only one event was considered as “possibly related” to the study panel. No safety signals were indicated.

12.2.4 Listing of Adverse Events by Subject

AEs are appended for individual subjects (see Appendix 16.2.7).

12.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

A serious AE was defined as any AE that resulted in death, a life-threatening event, required hospitalization or prolonged an existing hospitalization, caused a persistent or significant disability/incapacity, or resulted in a congenital anomaly or birth defect. Other significant events were defined as any AE that led to an intervention, including discontinuation from the study, or the need for treatment with significant additional concomitant medication.

12.3.1 Listing of Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

12.3.1.1 Deaths

No deaths were reported in the study.

12.3.1.2 Other Serious Adverse Events

No other serious AEs were reported in the study.

12.3.1.3 Other Significant Adverse Events

There were no significant AEs, and all subjects completed the study.

12.3.2 Narratives of Deaths, Other Serious Adverse Events, and Certain Other Significant Adverse Events

Not applicable; there were no deaths, other serious AEs, or significant AEs reported during the study.

12.3.3 Analysis and Discussion of Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

Not applicable; there were no deaths, other serious AEs, or significant AEs reported during the study.

12.4 Clinical Laboratory Evaluation

No clinical laboratory evaluations were conducted in this study.

12.5 Vital Signs, Physical Findings, and Other Observations Related to Safety

No vital sign measurements were obtained and no physical examinations were performed. However, the safety evaluation included the frequency of patch test reaction scores, the evaluation of tape-induced irritation at the test sites, subject-reported sensations of itching and burning, evaluations of late and persistent skin reactions, and reports of erythema, infiltration, hyperpigmentation, hypopigmentation, pruritus, and other reactions. Outcomes for these observations follow.

12.5.1 Frequencies of Patch Test Reaction Scores

The frequencies of all patch test reaction scores (positive [+1, +2, and +3], negative, irritant, and doubtful) are described in Section 11.4.1. Overall, the reference allergen elicited more reactions than any of the test concentrations of the T.R.U.E. TEST allergen. No irritant reactions occurred, and no positive reactions were elicited by the negative control.

12.5.2 Evaluation of Tape-Induced Irritation, and Itching and Burning

The number and frequency of subjects who reported none, weak, moderate, and strong tape irritation (Table 12.5.2.-1) and who reported itching and burning upon patch test removal were evaluated (Table 12.5.2-2). Of the 22 enrolled subjects, most experienced no tape-induced irritation to the investigational panel (14 or 63.6%) or chamber (13 or 59.1%). Weak tape-induced irritation was experienced by 8 subjects (36.4%) in response to the investigational panel and by 9 subjects (40.9%) in response to the chamber. None of the subjects experienced moderate or strong irritation in response to either the investigational panel or chamber. More than half the subjects reported that none to weak itching was associated with both the investigational panel (17 subjects or 77.3%) and the chamber (13 subjects or 59.1%). Fewer subjects reported that moderate to strong itching was associated with the investigational panel (5 subjects or 22.8%) compared to the chamber (9 subjects or 41%). Most patients reported no burning in response to either the investigational panel or chamber (17 subjects or 77.3% in both cases). In both cases, 3 subjects (13.6%) reported weak burning while 2 subjects (9.1%) reported moderate burning. No patients reported strong burning.

Table 12.5.2-1 Frequency of Tape Irritation Scores

	Investigational Panel								Chamber							
	None		Weak		Moderate		Strong		None		Weak		Moderate		Strong	
Analysis Population	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	14	63.6	8	36.4	0	0.0	0	0.0	13	59.1	9	40.9	0	0.0	0	0.0
PP Population	12	60.0	8	40.0	0	0.0	0	0.0	11	55.0	9	45.0	0	0.0	0	0.0

See SOURCE: Table 14.2.1
KEY TO SCORING TAPE IRRITATION
None: No irritation
Weak: Faint to definite pink erythema
Moderate:Moderate erythema, definite redness
Strong: Severe erythema, very intense redness

Table 12.5.2-2: Summary of Itching and Burning After Patch Removal (ITT Safety Population and Per Protocol Population)

		Investigational Panel								Chamber							
		None		Weak		Moderate		Strong		None		Weak		Moderate		Strong	
Analysis Population		**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ITT (Safety) Population	Itching	7	31.8	10	45.5	4	18.2	1	4.6	5	22.7	8	36.4	8	36.4	1	4.6
	Burning	17	77.3	3	13.6	2	9.1	0	0.0	17	77.3	3	13.6	2	9.1	0	0.0
PP Population	Itching	6	30.0	10	50.0	4	20.0	0	0.0	4	20.0	8	40.0	8	40.0	0	0.0
	Burning	16	80.0	3	15.0	1	5.0	0	0.0	16	80.0	3	15.0	1	5.0	0	0.0

See SOURCE: Table 14.2.13
KEY TO SCORING:
None: No itching or burning sensations
Weak: Minimal discomfort
Moderate:Definite discomfort
Strong: Significantly bothersome; possible interference with sleep or daily activity

12.5.3 Evaluations of Late and Persistent Skin Reactions

The number and frequency of late and persistent skin reactions were tabulated for all subjects (ITT safety population). A late reaction was defined as a positive test site skin reaction that initially occurred 7-10 days after application of the patch tests. No late reactions were reported for any subject in the study to any of the allergen test doses, to the negative control, or to the reference allergen.

A persistent reaction was defined as a positive test site skin reaction that appeared at Day 2 and/or Day 3 and/or Day 4 and persisted through Day 7 or to Day 21. Persistent reactions were further classified as healing or escalating. Overall, 18 of the 22 subjects (81.8%) had a persistent skin reaction to at least one of the allergen test doses or to the reference allergen or to both (see Listing 16.2.7). Fourteen patients (63.6 %) had a persistent reaction to the 0.40 mg/cm^2 dose: 11 (50%) were healing and 3 (13.6%) were escalating. Twelve subjects (54.5%) had a persistent reaction to the 0.20 mg/cm^2 dose: 10 (45.5%) were healing and 2 (9.0%) were escalating. Nine subjects (40.8%) had a persistent reaction to the 0.10 mg/cm^2 dose: 7 (31.8%) were healing and 2 (9.0%) were escalating. Seventeen subjects (77.2%) had a persistent reaction to the reference

allergen: 14 (63.6%) were healing and 3 (13.6%) were escalating (Table 14.3.1.9). At Visit 5 none of the patients had a persistent reaction to any allergen or to the negative control (Table 14.3.1.10 and Listing 16.2.7).

12.5.4 Hyperpigmentation, Hypopigmentation, Pruritis, and Other Reactions

For each T.R.U.E. TEST allergen concentration, the reference allergen, and the negative control, the number and frequency of subjects who reported hyperpigmentation, hypopigmentation, pruritus, or other features were tabulated for the ITT safety population for Visits 4 and 5 (Table 14.3.1.9 and Table 14.3.1.10).

Overall, the frequency of reactions characterized by pruritis and other characteristics was low, and there were no cases of hyperpigmentation or hypopigmentation (Listing 16.2.7, Table 14.3.1.9 and Table 14.3.1.10). None of the subjects reacted to the negative control. Furthermore, none of the subjects experienced hyper- or hypopigmentation in response to any patch test. Three subjects (13.6%) had pruritis in association with the 0.40 mg/cm^2 dose of the T.R.U.E. TEST allergen; one of these subjects also developed scaling. One subject (4.5%) had pruritis in association with the 0.20 mg/cm^2 dose of the T.R.U.E. TEST allergen; this patient was also one of the three subjects with pruritis in association with the 0.40 mg/cm^2 dose. Two subjects (9.1%) had pruritis in association with the 0.10 mg/cm^2 dose of the T.R.U.E. TEST allergen; one of these subjects did not have pruritis in association with either of the higher doses. Four subjects (18.2%) had pruritis in association with the reference allergen while two subjects (18.2%) had scaling in association with the reference allergen. All the subjects who developed pruritis or scaling were female.

12.6 Safety Conclusions

This study evaluated the safety of three concentrations of Lyral® within T.R.U.E. TEST patches. A summary of the safety results follows.

- There were no serious or severe AEs reported during the study, and none of the 22 enrolled subjects discontinued the study because of an AE.
- Three AEs were reported during the study, all of which were considered mild. There was one case of conjunctivitis, which was considered unrelated to the test protocol. There were two cases of pruritis involving an application site, one of which was considered possibly related to treatment and one of which was not considered related to treatment. In all three cases, the AE resolved.
- No patient had a late reaction to any of the patch tests. Overall, 18 of the 22 subjects (81.8%) had a persistent skin reaction to at least one of the allergen test doses or to the reference allergen or to both. More subjects had persistent reactions to the reference allergen than to the test doses of the allergen.
- No patient experienced hypo- or hyperpigmentation, and the overall frequency of pruritis was low.
- The majority of subjects experienced no or weak tape irritation in association with the test panel and the reference allergen.
- The majority of subjects experienced no or weak itching or burning in association with the test panel and the reference allergen.
- Overall, based on review of the AEs, tape-induced reactions, burning and itching reactions upon patch removal, and late and persistent skin reactions, no safety signals or trends appear to have been associated with the allergen formulations or control patches.

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13. DISCUSSION AND OVERALL CONCLUSIONS

Petrolatum allergens are used in standard diagnostic patch testing for ACD. However, allergen composition, test conditions, patient sensitivity, technical factors, and physician experience among other factors introduce considerable variability into the process. In turn, this variability can influence the intensity or skin reactions obtained and hence how the patch test reactions are interpreted.[1,2,3] In contrast, T. R.U.E. TEST is a ready-to-use patch test method designed for use by licensed physicians in the diagnosis of ACD. T.R.U.E. TEST has been evaluated in several large, multicenter clinical studies and is the only combined allergen and patch panel/chamber product currently approved by the Food and Drug Administration in the United States.

To continue to improve the efficiency of T.R.U.E. TEST for diagnosing ACD, it is important to expand the number of test allergens. To that end, the current study was designed to evaluate the diagnostic performance of three different concentrations of Lyral® T.R.U.E. TEST allergens in approximately 20 adult subjects with clinical histories of contact dermatitis and positive patch test results to Lyral® in petrolatum or to Fragrance mix 2 with the aim of determining the optimum dose compared to a reference standard of Lyral® 5% in petrolatum.

In this study, 22 subjects who were sensitive to Lyral®, 18 of whom were also sensitive to Fragrance mix 2, were enrolled at one investigational site. All 22 subjects completed the study and were compliant with the 2-day patch occlusion period. The 22 enrolled subjects had a mean (STD) age of 49.6 (11.8) years and were primarily female (77.3%) and Scandinavian (95.5%). Upon entry into the study, all subjects (100%) had previously been diagnosed with contact dermatitis and 9 (40.9%) subjects had active dermatitis with symptoms of dermatitis on their arms and/or hands.

The primary endpoints in this study included assessment of the optimal test allergen dose by determining the lowest concentration of Lyral® to elicit either +1 or +2 positive reactions in 70-90% of the enrolled subjects all of whom were considered “sensitive”; the frequency of positive, negative, doubtful, and irritant test site skin reactions for each allergen concentration; and the overall concordance and discordance between each test dose of the Lyral® T.R.U.E. TEST and the reference allergen as well as among the three doses of the Lyral® T.R.U.E. TEST allergens themselves.

Based on the investigator’s determination of positive responses, the greatest proportion of subjects in the PP population who had a positive reaction to Lyral® on the T.R.U.E. TEST panel was 70% at the highest concentration of 0.40 mg/cm^2. Thus, this was the only concentration of the test allergen that elicited a positive response from at least 70% of the sensitive subjects to meet the criteria for the optimal dose. In contrast, the reference allergen of Lyral® 5% in petrolatum elicited 91% positive reactions (20 of 22 responses were positive in the ITT safety

population). Hence, the reference allergen was more efficacious than any of the T.R.U.E. TEST doses of allergen in eliciting positive reactions.

The reported concordance between T.R.U.E. TEST and petrolatum allergens averages around 64% but has ranged from 18% to 81%.[4,5] In this study, the Cohen's Kappa values ranged from 0.313 to 0.466, indicating fair to moderate agreement between the Lyral® doses and the reference allergen in petrolatum for the ITT safety population across visits. Based on the investigator's determination of positive reactions, the agreement between the T.R.U.E. TEST doses of allergen and the reference allergen was also fair to moderate. When the three Lyral® doses were compared to each other across visits, the values of Kappa ranged from 0.567-0.820 indicating moderate to very good agreement. In other words, the three different doses were associated with similar results. When the Lyral® doses were compared to each other in terms of the investigator's determination of positive reactions, the concordance increased, ranging from 0.718-0.908 and again indicating substantial agreement among the doses.

For the ITT safety population at Visits 3 and 4, the GEE analysis indicated a significant overall difference between treatments. The results of the multiple comparison analysis indicated a difference between the Lyral® doses 0.40, 0.20, and 0.10 mg/cm2 and the negative control. Additionally, the Lyral® 0.40 dose was statistically different than the Lyral®0.10 dose. Based on the investigator's determination of positive reactions, the GEE analysis indicated a significant overall difference between treatments. The results of the multiple comparisons analysis indicated a difference between the Lyral® doses (0.40, 0.20, and 0.10 mg/cm2) and the negative control.

Overall, the results suggest that none of the three doses of T.R.U.E. TEST Lyral® allergen can likely be considered clinically relevant, a conclusion supported by the lack of concordance between the three test doses of Lyral® and the reference allergen in petrolatum.

In this study, no subject experienced a serious or severe AE and no subject discontinued because of an AE. Of the 22 subjects enrolled in the study, three subjects reported one AE each, all of which were considered mild and nonserious. Two events were reported by female subjects and one was reported by a male subject. The first event was reported as "conjunctivitis." The other two events, one of which was deemed "possibly related" to the study panel, were reported as pruritis at the application site. All events resolved without complications.

No patient had a late reaction to any of the patch tests. Overall, 18 of the 22 subjects (81.8%) had a persistent skin reaction to at least one of the allergen test doses or to the reference allergen or to both. More subjects had persistent reactions to the reference allergen than to the test doses of the allergen, further supporting the safety the test allergens. Furthermore, no patient experienced hypo- or hyperpigmentation, and the overall frequency of pruritis was low. The majority of subjects experienced no or weak tape irritation and no or weak itching and burning in association with the test panel and the reference allergen. Overall, based on review of the AEs, tape-induced reactions, burning and itching reactions upon patch removal, and late and persistent skin

reactions, no safety signals or trends appear to have been associated with the allergen formulations or control patches.

In conclusion, the results of this study indicate that the reference allergen in petrolatum was more efficacious in eliciting positive skin reactions than the Lyral® T.R.U.E. TEST allergen. Based on the investigator's determination of positive responses, the greatest proportion of subjects in the PP population who had a positive reaction to Lyral® on the T.R.U.E. TEST panel was 70% at the highest concentration of 0.40 mg/cm^2. Thus, this was the only concentration of the test allergen that elicited a positive response from at least 70% of the sensitive subjects to meet the criteria for the optimal dose. Based on a review of AEs; the frequency of patch test reaction scores; the evaluations of irritation, burning, and stinging; the evaluations of late and persistent skin reactions; and the reports of hyperpigmentation, hypopigmentation, pruritus, and other reactions, the safety results indicate that the Lyral® T.R.U.E. TEST allergen was well tolerated at all concentrations when applied under occlusion for two days.

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14. TABLES, FIGURES, AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

The following source documents from the data analysis are appended:

SP 12 1 PL 201 - Final stat Report 10-29-2013 (1).docx

SP 12 1 PL 201 Demographics Tables 26SEP13.rtf

SP 12 1 PL 201 Irritation and Adhesion Tables 28OCT13.rtf

Corrected SP 12 1 PL 201 Safety Tables 18JAN14.rtf

Corrected Listings SP 12 1 PL 201 Listings 20JAN14.rtf

14.1.1 Listings of Deaths, Other Serious, and Significant Adverse Events

Not applicable; there were no deaths, other serious AEs, or other significant AEs reported in this study.

See SOURCE: Table 14.3.1.6 and Table 14.3.1.7

14.1.2 Narratives of Deaths, Other Serious, and Certain Other Significant Adverse Events

Not applicable; there were no deaths, other serious AEs, or other significant AEs reported in this study.

14.1.3 Abnormal Laboratory Value Listing

Not applicable; no clinical laboratory assessments were conducted in this study.

15. REFERENCE LIST

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16. APPENDICES

16.1 Study Information

16.1.1 Protocol and Protocol Amendments

The final protocol is appended (see Final Protocol December 17 2012.pdf). There were no amendments or changes to the planned conduct of the study.

16.1.2 Sample Case Report Form

A sample CRF is appended (see Lyral Source-CRF.doc).

16.1.3 List of Independent Ethics Committees or Institutional Review Boards; Representative Written Information for Subjects, Including Sample Consent Forms

The IEC and IRB utilized by the investigational sites in Denmark and the US, respectively, follow. A representative copy of the informed consent document is presented after the table.

IEC or IRB	Address
University of Southern Denmark Ethics Committee	Institute of Clinical Research, Department of Dermoto-Venerology and Allergy Center Sdr. Boulevard 29 DK-5000 Odense C, Denmark

16.1.4 List and Description of Investigators and Other Important Participants in the Study, Including Brief *Curricula Vitae* or Equivalent Summaries of Training and Experience Relevant to the Performance of the Clinical Study

The investigators and sub-investigators who participated in this study were as follows:

Investigator (Sub-investigator)	**Address**
Evy Paulsen, Ph.D. and (Charlotte G. Mørtz, Ph.D.; Klaus Ejner Andersen, M.D.)	Institute of Clinical Research, Department of Dermoto-Venerology and Allergy Center Sdr. Boulevard 29 DK-5000 Odense C, Denmark Telephone: +45 65 41 27 08

Their curricula vitae are appended:

Evy Paulsen CV 2012.pdf

Charlotte Mortz CV 2012.pdf

Klaus Andersen CV 2012.pdf

16.1.5 Signature of Principal or Coordinating Investigator(s) or Sponsor's Responsible Medical Officer

STUDY TITLE (Protocol Number):

Final Report for Protocol SP 12 1PL 201: Clinical Evaluation of Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyral®) Dose Response Study

STUDY AUTHOR(S):

James P. Bowman, M.S.	Biostatistics
Shelley A. Kick, Ph.D.	Medical Writing

I have read this report and confirm that to the best of my knowledge, it accurately describes the conduct and results of the study.

Investigator or Sponsor's Responsible Medical Officer:

Curt Hamann, M.D. **Medical Director, Allerderm (dba) SmartPractice**	**Date**

See appended signed form for sponsor's responsible medical officer.

16.1.6 Listing of Subjects Receiving Investigational Product(s) from Specific Batches, Where More than One Batch was Used

Only one batch of investigational product was used in this study.

16.1.7 Randomization Scheme and Codes (Subject Identification and Treatment Assignments)

All T.R.U.E. TEST patches were included on each allergen panel, but in different configurations (see Configuration Lyral Dose Response Study.pdf). Subjects were assigned patches with randomized configurations (see Signed randomization list.pdf).

16.1.8 Audit Certificates

Not applicable; no investigational site audits were conducted.

16.1.9 Documentation of Statistical Methods

The statistical analysis plan is appended (see Lyral 12 1PL1 201 Signed SAP.pdf).

16.1.10 Documentation of Inter-laboratory Standardization Methods and Quality Assurance Procedures

Not applicable; no clinical laboratory evaluations were conducted for this study.

16.1.11 Publications Based on the Study

Not applicable; at the time of this writing, no publications have been produced using the data, results, or conclusions from this study.

16.1.12 Important Publications Referenced in the Report

Literature papers cited within this report follow.

16.2 Subject Data Listings

16.2.1 Discontinued Subjects

See the following appended listings:

SOURCE: Listing 16.2.3: Subject Disposition: ITT Safety Population

SOURCE: Listing 16.2.16: Inclusion Criteria: ITT Safety Population

SOURCE: Listing 16.2.17: Exclusion Criteria: ITT Safety Population

16.2.2 Protocol Deviations

At the prinicipal investigator's discretion, two subjects (Subjects 107 and 122) were enrolled into the study despite taking oral methotrexate (15 mg/week) for preexisting eczema in violation of the third exlusion criterion. Both subjects completed the study, but their data were only included in the safety analysis. No other protocol deviations were reported during the study (SOURCE: Listing 16.2.11).

16.2.3 Subjects Excluded from the Efficacy Analysis

All enrolled subjects (ITT safety population) were included in the analyses (SOURCE: Listing 16.2.10) with the exception of Subjects 107 and 122 who were excluded from the per protocol analysis due to violation of the third exclusion criterion (SOURCE: Listing 16.2.12).

16.2.4 Demographic Data

See the following appended listings:

SOURCE: Listing 16.2.1: Demographic Information: ITT Safety Population

SOURCE: Listing 16.2.2: Current Dermatitis Symptoms and Type of Dermatitis

SOURCE: Listing 16.2.9: Previous Patch Test Results: Lyral

SOURCE: Listing 16.2.9: Previous Patch Test Results: Fragrance Mix 2

SOURCE: Listing 16.2.15: Medical History and Concomitant Medications: ITT Safety Population

16.2.5 Compliance and/or Drug Concentration Data

See the following appended listings:

SOURCE: Listing 16.2.4: Panels Application and Removal Data and Tape Irritation Scores: ITT Safety Population

SOURCE: Listing 16.2.8: Adhesion Scores, Factores and Skin Condition: ITT Safety Population

16.2.6 Individual Efficacy Response Data

See the following appended listings:

SOURCE: Listing 16.2.5: Skin Reactions: ITT Safety Population Visit 3 Day 4

SOURCE: Listing 16.2.5: Skin Reactions: ITT Safety Population Visit 4 Day 7

SOURCE: Listing 16.2.5: Skin Reactions: ITT Safety Population Visit 5 Day 21

SOURCE: Listing 16.2.6 Investigator's Determination of Positive Reactions: ITT Safety Population

SOURCE: Listing 16.2.7: Late and Persistent Reactions: ITT Safety Population Visit 4 Day 7

SOURCE: Listing 16.2.7: Late and Persistent Reactions: ITT Safety Population Visit 5 Day 21

16.2.7 Adverse Event Listings by Subject

See the following appended listing:

SOURCE: Listing 16.2.14: Adverse Events: ITT Safety Population

16.2.8 Listing of Individual Laboratory Measurements by Subject

See the following appended listing:

SOURCE: Listing 16.2.13: Urine Pregnancy Test Results: ITT Safety Population Case Report Forms

16.2.9 Case Report Forms for Deaths, Other Serious Adverse Events, and Withdrawals for Adverse Events

Not applicable; there were no deaths, other serious AEs, or withdrawals due to AEs in this study.

16.2.10 Other Case Report Forms Submitted

Not applicable; no CRFs are included within this report, but all CRFs are available upon request.

16.3 Individual Subject Data Listings

Not applicable; no subject profiles were produced.