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Clinical Evaluation of Metal Panel Allergens: Aluminum, Copper, Manganese, Molybdenum, Tin, Titanium, Vanadium and Zinc Dose Response Study

Sponsor Study Number:	SP 14 8MP 201
NCT Number:	NCT02615249
IND Number:	16775
EudraCT Number:	2015-002678-19
PMDA Number:	P3726
Protocol Version:	Amendment V
Protocol Date:	27 June 2018

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CLINICAL PROTOCOL
CONFIDENTIAL

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2.0 APPROVAL OF THE AMENDED PROTOCOL

The Investigator(s) agree to conduct the trial as outlined in this protocol with reference to national/local regulations and in accordance with ICH Good Clinical Practice (GCP) guidelines. Any modification to the protocol must be approved in writing by the investigator, the sponsor, Health Authorities and the Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) as required by national regulations.

The Investigator(s) agree, by written consent to this protocol, to fully co-operate with monitoring and audit checks by allowing direct access to subject's study data and records, including source data, by authorised individuals representing sponsor or Health Authorities.

Approved consent in writing:

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3.0 TABLE OF CONTENTS

4.0 SUMMARY

4.1	Clinical Study Title	Clinical Evaluation of Metal Panel Allergens: Aluminum, Copper, Manganese, Molybdenum, Tin, Titanium, Vanadium and Zinc Dose Response Study
4.2	Clinical Study Number	SP 14 8MP 201
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	IRB/Ethics Committees *(continued)*	Clinical Trial Center for Developmental Therapeutics Tokushima University Hospital 2-50-1 Kuramoto-cho 770-8503 Tokushima, Japan Tel: +81-88-633-9294 Fax: +81-88-633-9295 Toho University Omori Medical Center Institutional Review Board Toho University Omori Medical Center 6-11-1, Omori-Nishi, Ota-ku Tokyo 143-8541 Japan
4.6	Objective	To determine the optimal test allergen dose of metal allergens proposed for inclusion in Metal Panel T.R.U.E. TEST. The study will compare the diagnostic performance (primary endpoint) and safety (secondary endpoint) of ascending patch test doses of aluminum, copper, magnesium, molybdenum, tin, titanium, vanadium and zinc allergens. To determine if subjects who have not had previous patch testing for contact metal allergy are allergic to any of the most common metal allergens; nickel, cobalt, chromium and gold.
4.7	Study Design	This is a prospective, multi-center, randomized, double-blind design. That is, the allergen doses on each panel will be randomized into three different configurations, which will be randomly assigned to subjects as they enter the study. Although the investigators and subjects will know which allergen is being tested, they will be blinded to the placement of the allergen doses within each panel. A 48-hour application (approximate) of investigational allergen panel(s), excipient controls and corresponding reference petrolatum (or aqueous) allergen(s) will be applied to the skin of human subjects to test for potential positive allergic responses. Test sites will be evaluated at 3-4, 7-8, 10-14 and 19-23 days after application.
4.8	Primary and Secondary Endpoints	*Primary Endpoint* Determination of optimal test allergen dose as: ▪ The lowest concentration of each dilution series allergen eliciting positive responses in a minimum of 15 subjects. Positive responses are defined as score of 1+, 2+ or 3+ during at least one reaction assessment visit. If a significant number of 3+ responses are elicited, the dose will be selected based on 1+ and 2+ responses. ▪ For all sites with the exception of Germany: Concordance will be measured using Cohen's kappa where less than 0% indicates no agreement, 0-20% indicates poor agreement, 20-40% indicates fair agreement, 40-60% indicates moderate agreement, 60-80% indicates good agreement and 80% or higher indicates very good agreement.

	Concordance will be measured using all subjects who are tested with each allergen and corresponding reference allergen. *Secondary Endpoint* Determination of allergen safety: ▪ Frequency of tape and polyester chip induced irritation or allergic reactions at Visits 2 through 6. ▪ Frequency of subject reported sensations of itching and/or burning for each allergen panel at patch removal. ▪ Frequency of positive (1+, 2+, 3+) skin reactions for each investigational and reference allergen dose at each post removal visit and overall. ▪ Frequency of negative, doubtful, irritant, late and persistent skin responses for each investigational and reference allergen dose at each post removal visit (late and persistent reactions at visits 4, 5 and 6 only). ▪ Frequency of all adverse events. Documentation for all local and systemic adverse reactions classified by the investigator as possibly or definitely related to the study product (e.g., erythema, hyper-pigmentation, hypo-pigmentation, skin thinning or dermatitis flare) will include grade (mild, moderate or severe) and time point (clinic visit). Positive results from the common allergen testing will be tabulated in the final report only. No additional analysis will be performed using this data. Comparative data analyses of reactions to these allergens are beyond the scope of this study.
4.9 Subjects	The study population will consist of subjects with past positive patch test result to at least one of the dilution series metals being tested on this study or strong suspicion of metal contact allergy. A minimum of 15 subjects per dilution series allergen, who exhibit a positive skin response (score of 1+, +2 or 3+ during at least one reaction assessment visit) to the dilution series allergen and/or at least one of its corresponding reference allergens, is needed to complete the study. Subjects with a past positive patch test response to at least one of the dilution series allergens will be tested with the allergen panel and corresponding reference allergen(s) to which they have had the previous response. Subjects with suspicion of metal contact allergy will be tested with all dilution series and reference allergens. All subjects will be tested with the excipient controls. The common allergens will be tested at the discretion of the investigator.

		The study population should include a reasonable representation of patients who have undergone a metal replacement procedure.
4.10	Justification of Sample Size	Past dose response study populations have included 20 adult subjects (per allergen) with a historical positive patch test to the corresponding reference allergen. In these studies, determination of the optimal test allergen dose was the lowest concentration eliciting a 1+, 2+ or 3+ positive reaction in 70-90% of subjects with the fewest number of 3+ reactions; therefore a minimum of 14 subjects with positive reactions was needed to determine optimal test allergen dose. Due to the fact that not all of the metals being tested on this study have a large database of patients with past patch-test positive reactions, the inclusion criteria was modified to include subjects with a suspicion of metal allergy, in addition to those with a historical positive patch test. Because it is anticipated that not all subjects will test positive, the study will conclude when a total of 400 subjects have been tested whether or not the 15 positive response subjects per allergen quota is met. The option of further testing may be considered for any allergen that elicits a minimum of 8 positive responses. In such a case, the protocol will be amended
4.11	Investigational Allergen Panels	**Aluminum Dilution Series** 0.040 mg/cm² aluminum chloride hexahydrate 0.12 mg/cm² aluminum chloride hexahydrate 0.36 mg/cm² aluminum chloride hexahydrate 0.72 mg/cm² aluminum chloride hexahydrate 0.047 mg/cm² aluminum lactate 0.14 mg/cm² aluminum lactate 0.42 mg/cm² aluminum lactate 0.84 mg/cm² aluminum lactate **Copper Dilution Series** 0.013 mg/cm² copper sulfate anhydrous 0.040 mg/cm² copper sulfate anhydrous 0.080 mg/cm² copper sulfate anhydrous 0.12 mg/cm² copper sulfate anhydrous

Investigational Allergen Panels *(continued)*	**Manganese Dilution Series** 0.013 mg/cm² manganese chloride tetrahydrate 0.040 mg/cm² manganese chloride tetrahydrate 0.080 mg/cm² manganese chloride tetrahydrate 0.24 mg/cm² manganese chloride tetrahydrate **Molybdenum Dilution Series** 0.0067 mg/cm² ammonium molybdate 0.020 mg/cm² ammonium molybdate 0.040 mg/cm² ammonium molybdate 0.12 mg/cm² ammonium molybdate
	Tin Dilution Series 0.018 mg/cm² tin chloride dihydrate 0.037 mg/cm² tin chloride dihydrate 0.11 mg/cm² tin chloride dihydrate 0.33 mg/cm² tin chloride dihydrate **Titanium Dilution Series** **Titanium Citrate** Ammonium titanium peroxo citrate (0.055 mg Ti/cm²) Ammonium titanium peroxo citrate (0.11 mg Ti/cm²) Ammonium titanium peroxo citrate (0.22 mg Ti/cm²) **Titanium Lactate** Ammonium titanium lactate (0.070 mg Ti/cm²) Ammonium titanium lactate (0.14 mg Ti/cm²) Ammonium titanium lactate (0.28 mg Ti/cm²) **Titanium Oxide Oxalate** Potassium titanium oxide oxalate (0.060 mg Ti/cm²) Potassium titanium oxide oxalate (0.12 mg Ti/cm²) Potassium titanium oxide oxalate (0.24 mg Ti/cm²) Ammonium titanium oxide oxalate (0.055 mg Ti/cm²) Ammonium titanium oxide oxalate (0.11 mg Ti/cm²) Ammonium titanium oxide oxalate (0.22 mg Ti/cm²)

	Investigational Allergen Panels *(continued)*	**Vanadium Dilution Series** Vanadium chloride (0.0042 mg V/cm²) Vanadium chloride (0.0083 mg V/cm²) Vanadium chloride (0.025 mg V/cm²) Vanadium chloride (0.050 mg V/cm²) Vanadium oxide sulfate (0.0042 mg V/cm²) Vanadium oxide sulfate (0.0083 mg V/cm²) Vanadium oxide sulfate (0.025 mg V/cm²) Vanadium oxide sulfate (0.050 mg V/cm²)
		Zinc Dilution Series 0.013 mg/cm² zinc chloride 0.040 mg/cm² zinc chloride 0.080 mg/cm² zinc chloride 0.24 mg/cm² zinc chloride The dose per unit area of experimental T.R.U.E. Test allergens is calculated based on the molecular weight of the compound. Exceptions are Titanium and Vanadium allergens as molecular weights are not known for all of these, hence the dose per unit area for Titanium and Vanadium allergens is based only on the metal part of the compound.
		Common Allergens/ Excipient Controls 0.2 mg/cm^2 nickel sulfate 0.054 mg/cm^2 potassium dichromate 0.02 mg/cm^2 cobalt dichloride 0.075 mg/cm^2 gold sodium thiosulfate (GST) Blank patch Polyvinylpyrrolidone (PVP) Hydroxypropyl cellulose (HPC)
4.12	Reference Allergens	▪ The Aluminum Dilution Series will be tested concurrently with o Aluminum chloride hexahydrate, 10 % w/w in petrolatum o Aluminum lactate, 12 % w/w in petrolatum ▪ The Copper Dilution Series will be tested concurrently with copper sulfate anhydrous, 2 % w/w in petrolatum ▪ The Manganese Dilution Series will be tested concurrently with manganese chloride tetrahydrate, 2 % w/w in petrolatum ▪ The Molybdenum Dilution Series will be tested concurrently with ammonium molybdate, 1% aqueous solution. ▪ The Tin Dilution Series will be tested concurrently with tin chloride dihydrate, 1% w/w in petrolatum ▪ The Titanium Dilution Series will be tested concurrently with

		○ Ammonium titanium peroxo citrate, 17% w/w in petrolatum ○ Ammonium titanium lactate, 34% aqueous solution ○ Potassium titanium oxide oxalate, 22% w/w in petrolatum ○ Ammonium Titanium oxide oxalate 19% w/w in petrolatum ▪ The Vanadium Dilution Series will be tested concurrently with ○ Vanadium chloride, 1% w/w in petrolatum ○ Vanadium oxide sulfate, 1.5% w/w in petrolatum ▪ The Zinc Dilution Series will be tested concurrently with zinc chloride, 2% w/w in petrolatum NOTES: 1) The common allergens (Panel 6) will not be tested against reference allergens. 2) Subjects enrolled in Germany will not be tested with the reference allergens The Finn Chamber, polypropylene-coated, supplied by SmartPractice, will be used to apply the reference allergens. Reference allergens will be dispensed at the rate of 20 µl/chamber using a precision allergen dispenser.
4.13	Study Outline	After the subject has consented and all inclusion/exclusion criteria are met the subject may be eligible for enrollment. If enrolled, the following timeline will be followed:
	Clinic Visit 1 Day 0	A. Investigational allergen panel(s) and the appropriate reference allergen(s) will be applied to the skin on the subject's back. Patch tests are to be worn for approximately 48 hours.
	Clinic Visit 2 Day 2	B. Adhesion of the investigational allergen panel(s) and the reference allergen(s) will be evaluated. Tape and polyester chip induced site irritation will be scored. Subject reported itching and burning sensations will be captured. Adverse events will be documented.
	Clinic Visit 3 Day 3-4	C. Tape and polyester chip induced site irritation will be scored. Skin reactions will be evaluated. Adverse events will be documented. De-identified photographs of site reactions may be taken at the discretion of the Investigator and will not be reviewed or collected by the study sponsor.
	Clinic Visit 4 Day 7-8	D. Unresolved tape and or polyester chip induced irritation will be scored. Skin reactions will be evaluated. Late or persistent reactions and adverse events will be documented. De-identified photographs of site reactions may be taken at the discretion of the Investigator and will not be reviewed or collected by the study sponsor.
	Clinic Visit 5 Day 10-14	E. Unresolved tape and or polyester chip induced irritation will be scored. Skin reactions will be evaluated. Late or persistent reactions and adverse events will be documented. De-identified photographs of site reactions may be taken at the discretion of the Investigator and will not be reviewed or collected by the study sponsor.

	Clinic Visit 6 Day 19-23	F. Follow up visit to record unresolved tape or polyester chip induced irritation, late or persistent reactions and documentation of adverse events. Photographs of site reactions may be taken at the discretion of the Investigator. Investigator may elect to perform this evaluation via telephone if no reactions are present.
4.14	Monitored Parameters	*Visit 1 (Day 0)* ▪ Informed Consent ▪ Documentation of inclusion/exclusion criteria ▪ Completion of Qualification and Type of Metal Exposure Questionnaires ▪ Subject demographics (date of birth, gender and ethnicity) ▪ Concomitant medication use and medical history ▪ Urine pregnancy test (if needed) ▪ Physical examination to record current sites of dermatitis ▪ Application of the investigational allergen panel(s) and reference allergen(s) to the paraspinal region of the upper back. NOTE: Upper arms may be used if there is not adequate space on the back for all panels. *Visit 2 (Day 2)* ▪ Documentation of adhesion of investigational allergen panel(s) and reference allergen(s) to confirm adequate allergen-to-skin contact ▪ Removal of investigational allergen panel(s) and reference allergen chamber(s) ▪ At least 15-minute wait between patch removal and irritation evaluation. ▪ Evaluation of tape and polyester chip induced irritation. ▪ Documentation of subject self-report of itching and burning sensations at test panel sites ▪ Documentation of subject-reported adverse events, worsening of a pre-existing condition/disease/symptom or changes to concomitant medications. *Visit 3 (Day 3-4)* ▪ Evaluation of tape and polyester chip induced irritation. ▪ Evaluation of patch site skin reactions ▪ Documentation of subject-reported adverse events, worsening of a pre-existing condition/disease/symptom or changes to concomitant medications. ▪ Photographs of site reactions (taken at the discretion of the Investigator). *Visit 4 (Day 7-8)* ▪ Evaluation of unresolved tape and or polyester chip induced irritation.

	▪ Evaluation of patch site skin reactions ▪ Categorization of positive reactions as late or persistent ▪ Documentation of subject-reported adverse events, worsening of a pre-existing condition/disease/symptom or changes to concomitant medications. ▪ Photographs of site reactions (taken at the discretion of the Investigator). *Visit 5 (Day 10-14)* ▪ Evaluation of unresolved tape and or polyester chip induced irritation. ▪ Evaluation of patch site skin reactions ▪ Categorization of positive reactions as late or persistent; ▪ Documentation of subject-reported adverse events, worsening of a pre-existing condition/disease/symptom or changes to concomitant medications. ▪ Photographs of site reactions (taken at the discretion of the Investigator). *Visit 6 (Day 19-23)* ▪ The investigative site will phone the subject to determine if there are any unresolved reactions from the tape, or at the individual test sites. If the subject reports no remaining reactions the Investigator may choose at this point to complete the visit over the telephone. ▪ If the visit is conducted over the telephone all visit related paperwork including adverse events and/or changes to concomitant medications will be recorded. ▪ Should the Investigator elect to see the subject in the office, the following procedures will be performed: ▪ Evaluation of unresolved tape and or polyester chip induced irritation. ▪ Evaluation of patch site skin reactions. ▪ Categorization of positive reactions as late or persistent. If a persistent, escalating reaction is noted at Visit 6, the investigator will determine if follow up action is warranted. ▪ Subject-reported adverse events, worsening of a pre-existing condition/disease/symptom or changes to concomitant medications will be recorded. ▪ Photographs of site reactions (taken at the discretion of the Investigator). ▪ Following Visit 6 the Investigator will categorize the skin reactions at each site as either negative or positive.
4.15 Data Analysis	The frequency of skin responses; positive (1+, 2+, 3+), negative, doubtful and irritant, will be calculated for each dilution series investigational allergen dose and reference allergen. The optimal

	allergen dose will be based on the following criteria: ▪ The lowest concentration of each dilution series allergen eliciting positive responses in a minimum of 15 subjects. Positive responses are defined as score of 1+, 2+ or 3+ during at least one reaction assessment visit. If a significant number of 3+ responses are elicited, the dose will be selected based on 1+ and 2+ responses. ▪ For all sites with the exception of Germany: Concordance will be measured using Cohen's kappa where less than 0% indicates no agreement, 0-20% indicates poor agreement, 20-40% indicates fair agreement, 40-60% indicates moderate agreement, 60-80% indicates good agreement and 80% or higher indicates very good agreement. Concordance will be measured using all subjects who are tested with each allergen and corresponding reference allergen. Safety evaluations will be tabulated as follows: ▪ Frequency of tape and polyester chip induced irritation or allergic reactions at Visits 2 through 6 for the T.R.U.E. Test tape and polyester chips (Panels 1-6) and Finn Chamber tape (Reference allergen panels). ▪ Frequency of subject reported sensations of itching and/or burning for each allergen panel at patch removal. ▪ Frequency of positive (1+, 2+, 3+) skin reactions for each investigational and reference allergen dose at each post removal visit and overall. ▪ Frequency of negative, doubtful, irritant, late and persistent skin reactions for each investigational and reference allergen dose at each post removal visit (late and persistent reactions at visits 4, 5 and 6 only). ▪ Frequency of all adverse events. Documentation for all local and systemic adverse reactions classified by the investigator as possibly or definitely related to the study product (e.g., erythema, hyper-pigmentation, hypo-pigmentation, skin thinning or dermatitis flare) will include grade (mild, moderate or severe) and time point (clinic visit). Statistical processing will be performed using SAS® software.
4.16 Study Duration	The proposed study is intended to begin in 2016, no later than 6 months after approval of the final protocol by the IRB or Ethics Committee. Given the rarity of sensitization to these metals, the study may be concluded at an individual site if the Investigator believes that the patient data base has been exhausted. When this maximum number of subjects has been reached the sponsor of the study will inform the investigator that the study has been concluded at that site.

	The duration of participation for each enrolled subject will be approximately three weeks. The final report will be submitted to the Principal Investigator within 120 days of data base lock and to the FDA, European Commission and PMDA within 1 year of study completion.

5.0 FREQUENTLY USED ABBREVIATIONS AND ACRONYMS

ACD	Allergic Contact Dermatitis
AE	Adverse Event
BLA	Biologics License Application
CRF	Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HPC	Hydroxypropyl cellulose
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use
ICDRG	International Contact Dermatitis Research Group
IEC	International Ethics Committee
IR	Irritant Reaction
IRB	Institutional Review Board
MPA	Medical Products Agency
PVP	Polyvinylpyrrolidone, povidone, polyvidone (PVP)
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
T.R.U.E. Test	Thin-Layer Rapid Use Epicutaneous Test
UV	Ultraviolet

6.0 BACKGROUND AND RATIONALE

6.1 Metal Allergy

As a group, metals are the most common contact allergens. Contact allergy to metals such as nickel, cobalt, and chromium is prevalent in the general population. It is estimated that up to 17% of women and 3% of men are nickel allergic, and that about 1 to 2% are allergic to cobalt, chromium, or both. Metal-induced allergic contact dermatitis (ACD) is expressed in a wide range of cutaneous reactions following prolonged or repeated exposure to personal products such as cosmetics, tattoos, detergents, jewelry, piercing studs, leather goods, cell phones, clothing buttons, snaps, zippers, partial dentures, dental braces and restorations. Occupational exposure in the metal and construction industries is also a significant risk factor for metal allergy reports. Apart from the well-known significance of nickel, cobalt, chromium and gold in developing ACD, other metals such as aluminum, beryllium, copper, iridium, indium, mercury, palladium, platinum, rhodium, molybdenum, manganese, zinc, cadmium and titanium have been reported as increasing causes of skin hypersensitivity.

6.2 Aluminum

The use of aluminum exceeds that of all other metals, except iron, and is used in all segments of the world's economy. It weighs about one-third as much as steel or copper; is malleable, ductile,

and easily machined and cast; and has excellent corrosion resistance and durability. Some of the many uses for aluminum are in transportation (automobiles, airplanes, trucks, railcars, marine vessels, etc.), packaging (cans, foil, etc.), construction (windows, doors, siding, etc.), consumer durables (appliances, cooking utensils, etc.), electrical transmission lines and machinery.

Aluminum compounds have a wide range of uses in industrial, domestic, consumer and medicinal products. The following summarizes several uses (3):

- Aluminum alkoxides- varnish, cosmetics and pharmaceuticals
- Aluminum borate- glass and ceramics
- Aluminum carbonate- antacids
- Aluminum chloride- (anhydrous) rubber, lubricants and antiperspirants
- Aluminum chloride- (hexahydrate) deodorants, antiperspirants, cosmetics and astringents
- Aluminum hydroxide- stomach antacids, antiperspirants, dentifrices and cosmetics
- Aluminum isopropoxide- soap and paint
- Aluminum nitrate- antiperspirants
- Aluminum phosphate- stomach antacids
- Aluminum silicate- dental cement, antacids and food additives

Typical causes of sensitization to aluminum are injections and vaccines. Aluminum compounds have been widely used as adjuvants in prophylactic and therapeutic vaccines as they prolong the period of absorption and increase the immune response. The two main clinical features of aluminum sensitization are persistent granulomas and recurrent eczema. Aluminum allergies seem to be more common in pediatric patients than adults. Children with aluminum sensitivity have been reported to develop subcutaneous nodules at the sites of injection or excoriated papules at the sites of hyposensitization therapy.

Prolonged use of antiperspirant and topical medications containing aluminum may also cause sensitization to aluminum. Clinical manifestations of aluminum sensitization are axillary rashes and hand dermatitis.

Pruritus due to allergic conditions has been seen after use of toothpaste containing 30-40% of aluminum oxide.

Researchers have proposed that tattoo pigments containing aluminum can induce granulomatous reactions. In 87% of tattoo ink studies, the most commonly identified element was aluminum.

6.3 Copper

Copper is a ductile metal with very high thermal and electrical conductivity. It is used as a conductor of heat and electricity, a building material, and a constituent of various metal alloys.

The major applications of copper are in electrical wires (60%), roofing and plumbing (20%) and industrial machinery (15%). Copper is mostly used as a pure metal, but when a higher hardness is required it is combined with other elements to make an alloy (5% of total use) such as brass and bronze.

Numerous copper alloys exist, many with important uses. Brass is an alloy of copper and zinc. Bronze usually refers to copper-tin alloys, but can refer to any alloy of copper such as aluminum bronze. Copper is one of the most important constituents of carat silver and gold alloys and carat solders used in the jewelry industry and the alloy of copper and nickel, is used in low-denomination coins. Copper is used in dental alloys and in intrauterine devices. Copper is also used in wood preservatives, antimicrobial textile treatments, ceramic glazes, stained glass, musical instruments and electroplating. Copper is also found naturally in foods but may also be introduced during preparation.

6.4 Manganese

Manganese is a chemical element found as a free element in nature (often in combination with iron), and in many minerals. Manganese is a metal with important industrial metal alloy uses, particularly in stainless steels. It is used as a treatment for rust and corrosion prevention on steel and as industrial pigments. Manganese dioxide is used as the cathode (electron acceptor) material in zinc-carbon and alkaline batteries. The second large application for manganese is as alloying agent for aluminum. Corrosion-resistant aluminum/manganese alloys are used for most beverage cans. Manganese compounds have been used as pigments and for the coloring of ceramics, glass and some paints and as a reagent in organic chemistry. Manganese chloride is used in oral agents for an MRI of the abdomen and/or pelvis. Manganese oxide is used in plant food.

6.5 Molybdenum

Molybdenum, a chemical element, occurs in various oxidation states in minerals. The majority of molybdenum produced is used in metallurgical applications such as structural steel, stainless steel, tool & high-speed steels, cast iron, molybdenum elemental metal, and super alloys, with the remaining portion used as compounds in chemical applications. The ability of molybdenum to withstand extreme temperatures without significantly expanding or softening makes it useful in applications that involve intense heat, including the manufacture of armor, aircraft parts, electrical contacts, pigments, catalysts, industrial motors, and filaments. Molybdenum is also used as a fertilizer for some plants, as a solid lubricant, a high-pressure high-temperature anti-wear agent, an adhesive between enamels and metals, a coloring agent in ceramics and plastics as well as in biological staining procedures.

6.6 Tin

Tin is a silvery, malleable poor metal used to coat other metals to prevent corrosion. Tin is used in many alloys, most notably tin/lead soft solders, tin plating, tin chemicals, brass and bronze, solder and niche uses.
Tin is most commonly alloyed with copper and may be found in some coins. Tin is also found in some dental amalgams and fluoride treatments.

6.7 Titanium

Titanium (IV) oxide is used as pigment to color paints, sunscreens, cosmetics, skin care products, plastics, papers, inks, medicines, toothpastes, and foods such as milk. It is also a thickener found in tattoo pigment and styptic pencils. It has UV resistant properties and is therefore used to act as a UV absorber.

6.8 Vanadium

Vanadium is a hard, silvery gray, ductile and malleable transition metal used in applications for bicycle frames, cranks, axles, and gears. It is also found in orthopedic and dental implants, glass coatings and jewelry. It is mainly used to produce specialty steel alloys such as high speed tool steels. The most important industrial vanadium compound, vanadium pentoxide, is used as a catalyst for the production of sulfuric acid. Various vanadium oxides are also used in making ceramics, glass coatings and jewelry.

6.9 Zinc

Zinc is a metallic chemical element used to in the manufacture of cement, paper and textile processing it is an accelerator in the production of rubber. It is found as a pigment in paint and as a primer for galvanized iron and other metal substrates. Other applications include batteries, small non-structural castings, and alloys, such as brass (copper and zinc). A variety of zinc compounds are commonly used, such as zinc carbonate and zinc gluconate (as dietary supplements), zinc chloride (in deodorants), zinc pyrithione (antibacterial agent, anti-dandruff shampoos), zinc sulfide (in luminescent paints), and zinc methyl or zinc diethyl in the organic laboratory.

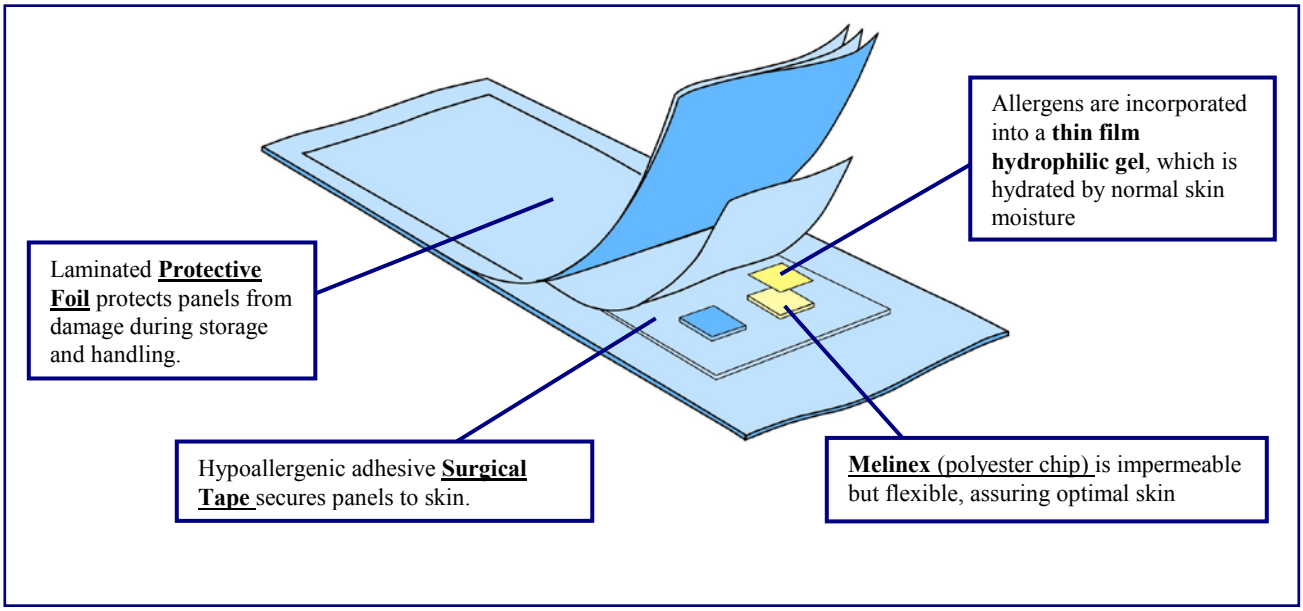
6.10 Patch Testing with Metals

A broad array of metals is available for patch testing today. These allergens are manufactured in a petrolatum, aqueous or alcohol based excipient and sold in syringes throughout the world although none have been submitted to the FDA or to the MPA in conformance with the Biologics License Application (BLA) or Medicinal Product Application procedures.

6.11 T.R.U.E. TEST®

T.R.U.E. TEST®, (Thin-layer Rapid Use Epicutaneous 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 in the U.S. population, 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 (control). 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), which are mounted on tape forming 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 at 72-96 hours after the application, when the allergic responses are fully developed and mild irritant reactions have faded. Additional readings at 1 week and 21 days after panel placement are also advised in some cases.

Figure 1: T.R.U.E. TEST® Panel



6.12 Metal Panel T.R.U.E. TEST

Today the T.R.U.E. TEST® contains the only patch test allergens that have been submitted to the FDA and approved for sale in the United States. As such, these allergens require extensive developmental and clinical trial work prior to submission for approval. The clinical trials require subjects with suspicion of contact allergy (possibly to metals) or local inflammation potentially associated with a metal implant to determine both the appropriate dose for the allergen as well as the safety & efficacy of the proposed product.

Because metal contact allergy is increasing there is a need to develop a series of standardized metal patch test allergens utilizing the same allergen delivery technology as used in the T.R.U.E. TEST® product. Metal Panel T.R.U.E. TEST will be indicated for patients exposed to cardiac implants (stent, pacemaker, etc.), orthopedic implants (knee, hip or other), gynecological implants or devices, surgical hardware (plates, screws, wires, pins, rods, expanders, staples), dental metal implants, or dental metal appliances, prosthesis or fillings whose exposure to has resulted in:

- Inflammation associated with an oral metal implant:
  - Burning mouth syndrome
  - Oral lichen planus
  - Oral lichenoid lesion
  - Palmo plantar pustulosis
  - Stomatitis: gingivitis, cheilitis and/or glossitis
- Itching, papules or nodules at injection site of aluminum containing vaccination
- Dermatitis over site of metal implant
- Systemic contact dermatitis
- Accelerated restenosis of cardiac stent
- Aseptic loosening
- Persistent joint pain

Other symptoms may also be associated:

- Persistent and recalcitrant dermatitis
- Dorsal or patchy hand dermatitis
- Leg and foot dermatitis
- Facial dermatitis (excluding seborrheic)
- Discoid dermatitis
- Dermatitis with unusual distribution
- Atypical allergic symptoms

In addition to the 8 metals on this study, is anticipated that Metal Panel T.R.U.E. Test will include metals currently available on T.R.U.E. TEST® and additional metals being investigated in a separate protocol.

- Nickel sulfate (currently on T.R.U.E. TEST®)

- Potassium dichromate (currently on T.R.U.E. TEST®)
- Cobalt dichloride (currently on T.R.U.E. TEST®)
- Gold sodium thiosulfate (currently on T.R.U.E. TEST®)
- Ammoniated Mercury (investigated in separate protocol)
- Sodium tetrachloropalladate (investigated in separate protocol)

The four common allergens (nickel, chromate, cobalt and gold) have been added to this protocol for diagnostic purposes only. Subjects with a strong suspicion of metal contact allergy should be tested with the most common metal allergens in addition to the 8 experimental (dilution series) metal allergens in order to give them a complete diagnosis. The common allergens will be tested as approved for T.R.U.E. Test but are considered experimental for this study due to reconfiguration of allergen placement on the test panel. Positive results from the common allergen testing will be tabulated in the final report only. No additional analysis will be performed using this data.

7.0 OBJECTIVE

To evaluate the diagnostic performance and safety of metal allergens proposed for inclusion in Metal Panel T.R.U.E. Test. The study will compare the diagnostic performance (primary) and safety (secondary) of ascending patch test doses of aluminum, copper, manganese, molybdenum, tin, titanium, vanadium and zinc allergens.

To determine if subjects who have not had previous patch testing are allergic to any of the most common metal allergens; nickel, cobalt chromium and gold.

7.1 Primary Endpoint

Determination of optimal test allergen dose as:

- The lowest concentration of each dilution series allergen eliciting positive responses in a minimum of 15 subjects. Positive responses are defined as score of 1+, 2+ or 3+ during at least one reaction assessment visit. If a significant number of 3+ responses are elicited, the dose will be selected based on 1+ and 2+ responses.
- For all sites with the exception of Germany: Concordance will be measured using Cohen's kappa where less than 0% indicates no agreement, 0-20% indicates poor agreement, 20-40% indicates fair agreement, 40-60% indicates moderate agreement, 60-80% indicates good agreement and 80% or higher indicates very good agreement. Concordance will be measured using all subjects who are tested with each allergen and corresponding reference allergen.

7.2 Secondary Endpoint

Determination of allergen safety

- Frequency of tape and polyester chip induced irritation or allergic reactions at Visits 2 through 6.

- Frequency of subject reported sensations of itching and/or burning for each allergen panel at patch removal.
- Frequency of positive (1+, 2+, 3+) skin reactions for each investigational and reference allergen dose at each post removal visit and overall.
- Frequency of negative, doubtful, irritant, late and persistent skin reactions for each investigational and reference allergen dose at each post removal visit (late and persistent reactions at visits 4, 5 and 6 only).
- Frequency of all adverse events. Documentation for all local and systemic adverse reactions classified by the investigator as possibly or definitely related to the study product (e.g., erythema, hyper-pigmentation, hypo-pigmentation, skin thinning or dermatitis flare) will include grade (mild, moderate or severe) and time point (clinic visit).

8.0 STUDY DESIGN

8.1 Overview

This is a prospective, multi-center, randomized, double-blind design. That is, the allergen doses on each panel will be randomized into three different configurations, which will be randomly assigned to subjects as they enter the study. Although the investigators and subjects will know which allergen is being tested, they will be blinded to the placement of the allergen doses within each panel. A 48-hour application (approximate) of investigational allergen panel(s), an excipient control and corresponding reference petrolatum (or aqueous) allergen(s) will be applied to the skin of human subjects to test for potential positive allergic responses. Test sites will be evaluated at 3-4, 7-8, 10-14 and 19-23 days after application. The chosen evaluation times are consistent with generally accepted international patch test guidelines and are designed to prevent missed late reactions and false negatives. The final clinic visit (day 21) allows the investigator to evaluate any late or persistent localized reactions. The investigator may choose to perform this visit via telephone if there are no residual reactions. If there are persistent escalating reactions noted at Visit 6, the investigator will determine and record follow up action.

8.2 Study Conduct

The study will be performed in accordance with the Helsinki Declaration (2013), the GCP-guidelines and regulatory requirements including Human Subjects and Privacy protection. Study subjects may be modestly compensated for their participation.

8.3 Independent IRB/ Ethics Committee Review

The study protocol, any amendments and the informed consent will receive favorable approvals from all regulatory authorities (US-FDA, Switzerland-Health Authority Swissmedic, Italy-Italian Medicines Agency, Netherlands-Medicines Evaluation Board, Germany-Paul Ehrlich Institut, Japan- PMDA) and from independent IRB/Ethics committees for each study site prior to study

initiation or prior to implementation of protocol changes. This study will not proceed without receipt of all written approvals.

8.4 Subject Information and Consent

Consent, including photograph consent, will be obtained prior to participation in any study conduct. Subjects will be given ample opportunity to read the consent form and have all questions regarding the study objective, procedures, possible risks, and the right to withdraw answered prior to signing and dating the consent form. Each subject will be provided with a signed and dated copy of the informed consent form to retain for his or her records.

8.5 Data Collection

Data will be collected using a data collection form, which will capture documented compliance with inclusion/exclusion criteria, medical history, current medication use, current dermatitis, panel adhesion, tape irritation, subject-reported itching and burning at patch sites, evaluation of skin patch site reactions and any changes to the subject's medical condition or medications used during the course of the study.

8.6 Study Duration

The proposed study is intended to begin in 2016, no later than 6 months after approval of the final protocol by the IRB or Ethics Committee. Given the rarity of sensitization to these metals, the study may be concluded at an individual site if the Investigator believes that the patient data base has been exhausted. When this maximum number of subjects has been reached the sponsor of the study will inform the investigator that the study has been concluded at that site.

The duration of participation for each enrolled subject will be approximately three weeks.

The final report will be submitted to the Principal Investigator within 120 days of data base lock and to the FDA, European Commission and PMDA within 1 year of study completion.

9.0 SUBJECTS

The study population will consist of subjects with past positive patch test result to at least one of the dilution series metals being tested on this study or strong suspicion of metal contact allergy.

A minimum of 15 subjects per dilution series allergen, who exhibit a positive skin response (score of 1+, +2 or 3+ during at least one reaction assessment visit) to the dilution series allergen and/or at least one of its corresponding reference allergens, is needed to complete the study.

Subjects with a past positive patch test response to at least one of the dilution series allergens will be tested with the allergen panel and corresponding reference allergen(s) (with the exception of subjects enrolled in Germany who will not be tested with the reference allergens) to which they have had the previous response. Subjects with suspicion of metal contact allergy will be

tested with all dilution series and reference allergens. All subjects will be tested with the excipient controls. The common allergens will be tested at the discretion of the investigator.

The study population should include a reasonable representation of patients who have undergone a metal replacement procedure.

9.1 Overview

Subjects will be recruited from patients who are currently visiting or have previously visited dermatology, allergy or similar medical practices and clinics for patch testing or evaluation of suspicion of metal contact allergy potentially associated with a metal implant. Sensitive subjects (those who have had a past positive patch test to at least one of the dilution series allergens) will be tested with the allergen panels(s) and corresponding reference allergen(s) (with the exception of subjects enrolled in Germany who will not be tested with the reference allergens) to which they have had a previous response. Those with suspicion of metal contact allergy potentially associated with a metal implant will be tested with all dilution series and reference allergens. All subjects will be tested with the excipient controls. The common allergens will be tested at the discretion of the investigator

All subjects (not identified by name) considered for study inclusion who sign the consent form must be recorded in the Subject Screening Log. If a subject declines to participate or fails inclusion/exclusion entry criteria the reason for the screen fail will be recorded in the Subject Screening Log. Subjects enrolled in the study will be assigned a unique subject identification code that will also include a site-specific identifier.

9.2 Entry/Eligibility Criteria

9.2.1 Inclusion Criteria

a. 18 years of age or older.
b. Past positive patch test result within the past 10 years (to one of the dilution series metals being tested on this study) or strong suspicion of metal contact allergy based on results of the Qualification Questionnaire.
c. Unable to become pregnant or willing to use an acceptable method of contraception to prevent pregnancy if female of childbearing potential;
    - Inability to become pregnant would include all male subjects and female subjects who are postmenopausal for at least 1 year, or surgically sterile- have had a hysterectomy, bilateral ovariectomy, uterine ablation or bilateral tubal ligation.
    - Acceptable methods of contraception include: 1) systemic birth control (i.e., oral contraceptives, skin patch, vaginal ring, implant, injection, or intrauterine device (IUD), which contains either a hormone or copper); 2) double barrier method (i.e., diaphragm, cervical cap, sponge, condom with spermicide); 3) IUD; 4) vasectomized partner; or 5)

abstinence from sexual intercourse. Subject must agree to use acceptable contraception for the duration of the entire study.
(Notes:
- *Cervical cap and abstinence from sexual intercourse will not be considered as acceptable methods of contraception for subjects enrolled in Japan*
- *A double-barrier method must be used for all subjects enrolled in Switzerland who are practicing non-systemic methods of birth control.*
- *Abstinence from sexual intercourse will not be considered an acceptable method of contraception for subjects enrolled in Switzerland)*

d. Understands and signs the approved Informed Consent form which is consistent with all institutional, local and national regulations.

9.2.2 Exclusion Criteria

a. Breastfeeding or pregnant (as determined by urine pregnancy test) or intending to become pregnant during the course of the study. Breastfeeding may be resumed upon completion of the study.
b. Topical treatment with corticosteroids or other immunosuppressive agents on or near the test area 14 days prior to inclusion through the end of the subject's participation in the study.
c. Systemic treatment with corticosteroids (equivalent to > 10 mg prednisone) or other immunosuppressive agents 14 days prior to inclusion through the end of the subject's participation in the study. Inhaled treatments and steroidal nose or eye drops are permitted.
d. Treatment with ultraviolet (UV) light (including tanning) during the 3 weeks prior to inclusion through the end of the subject's participation in the study.
e. Acute dermatitis outbreak or dermatitis on or near the test area on the back.
f. Known or suspected infection of the skin, joints or other site(s) associated with metal exposure
g. Condition such as; fibromyalgia, chronic fatigue, depression, cognitive impairment, flu-like symptoms, diarrhea and/or headache without at least one of the symptoms related to metal exposure listed in Section 10.1 under physical examination.
h. Condition such as; psoriasis, dermatitis herpetiformis, mycosis fungoides or cutaneous T-cell lymphoma that may confound the evaluation of allergic contact dermatitis.
i. Inability to comply with patch test study requirements including multiple return visits and activity restrictions (e.g., protecting test panels from excess moisture due to showering or vigorous activity).
j. Participation in a clinical trial of an investigational drug, treatment or device during this study or 3 weeks prior to inclusion in this study.
k. An opinion of the Investigator that deems the potential subject to be non-compliant, unable to return for study visits or complete the study as detailed in the protocol.

The following exclusion criteria will be required in Germany only:
l. Alcohol abuse as well as drug and/or medication abuse.
m. Severe psychiatric, psychological or neurological disorders.

n. Patients in any relationship or dependency with the sponsor and/or investigator.
o. General inflammatory as well severe acute and chronic inflammatory diseases.
p. Malignancy during the previous 5 years.
q. Completed or ongoing long-term treatment with tranquilizer or psycho active drug.

9.3 Subject Withdrawal and Compliance

Subjects may withdraw from the study at any time. However, subjects will not necessarily be withdrawn from the study for missing scheduled visits. Subjects will be contacted and outcomes documented as described in the clinical protocol and investigator brochure. If a subject misses two or more clinic visits he or she may be withdrawn from the study at the discretion of the Investigator. Data from withdrawn subjects may be used in the safety analysis and will remain anonymous.

The Investigator may withdraw a subject if deemed medically necessary, or if the subject can no longer meet study requirements. Any subject who is withdrawn from the trial will continue to receive normal standard of care. Subject withdrawal and reasons for withdrawal must be clearly described and documented in the subject's data collection form. Data from withdrawn subjects may be used in the safety analysis and will remain anonymous. Withdrawn subjects will be replaced.

Additional criteria for subject withdrawal include:

- Overreaction to an allergen. Defined as a response greater than Extreme Positive (3+). A severe reaction to the test tape adhesive or allergen patch that either causes the subject to remove the panels prior to the 48 hour return visit or is determined by the investigator to be significantly greater in severity than expected or as described in the Investigator's Brochure. Test panels will be immediately removed and reactions treated per standard medical guidelines.
- Unacceptable adverse events. Defined as development of severe itching and burning, a severe dermatitis flare-up or other adverse event that may be considered unacceptable.

9.4 Subject Activity Restrictions

During the first two days of the study subjects must:
- Keep test panels and test sites dry and protected from UV light.
- Avoid activities that can hinder patch adherence or interfere with skin reaction evaluations such as swimming, sunbathing, tanning, submersion bathing, strenuous activities that may cause excessive perspiration and the use of steam rooms or saunas. Subjects may shower but should avoid getting the patch sites wet.

During the entire 3-week test period, subjects are restricted from:
- Sunbathing and tanning to prevent the occurrence of photo-induced skin reactions.
- Use of new topical or personal care products to prevent a dermatitis flare-up and possible confounding of skin reactions.
- Use of prescription or over-the-counter topical products at patch sites after panel removal unless directed and monitored by the Investigator.

The Investigator may withdraw a subject if the subject does not meet the study requirements.

10.0 MONITORED PARAMETERS

Figure 2: Visit Schedule

	Day 0 Visit 1	Day 2 Visit 2	Day 3-4 Visit 3	Day 7-8 Visit 4	Day 10-14 Visit 5	Day 19-23 Visit 6*
Read and Sign Consent	X					
Study Eligibility Questions	X					
Urine Pregnancy Test	X					
Skin Examination	X					
Patch Application	X					
Adhesion Evaluation		X				
15-minute wait		X				
Tape Irritation Evaluation		X				
Itching and Burning		X				
Skin Reaction Evaluation			X	X	X	X
Possible Photographs of Patch Sites			X	X	X	X
Changes to Medications or Illness Questions		X	X	X	X	X

* May be phone call or office visit.

10.1 Visit 1: Day 0

Documentation of the following must occur prior to application of the investigational panel and reference allergen:

- Informed Consent
  - *(Note: Legal representative in addition to subject must sign consent form for subjects aged 18-19, enrolled in Japan.)*
- Subject interview including medical history, medication use and documentation of inclusion/exclusion criteria
- Dipstick urine pregnancy test for all females of childbearing potential (mouse anti-beta hCG antibody conjugated to colloidal gold and goat anti-alpha hCG antibody coated on the membrane)
- Subject demographics (date of birth, gender, ethnicity)
- Completion of Qualification Questionnaire and Type of Metal Exposure Questionnaire
- Physical examination and chart review to record current symptoms of dermatitis related to metal exposure. The physical examination will facilitate documentation of the following symptoms related to metal exposure:
  - Dermatitis over site of metal implant
  - Systemic contact dermatitis
  - Accelerated restenosis of cardiac stent
  - Aseptic loosening
  - Persistent joint pain, chronic swelling, instability and/or loosening, itching or burning
  - Burning mouth syndrome
  - Oral lichen planus
  - Oral lichenoid lesion
  - Palmo plantar pustulosis
  - Stomatitis: gingivitis, cheilitis and/or glossitis
  - Itching, papules or nodules at injection site of aluminum containing vaccination
  - Other

Age of diagnosis, distribution, severity and current medication use (including those being withheld for the duration of the study) will be captured for subjects who exhibit concurrent atopic dermatitis and irritant dermatitis.

Figure 3: Qualification Questionnaire

☐ Past Positive patch test result(s) within the past 10 years

Name of allergen tested: _____

Date of test: _____

Results: _____

Symptoms at time of patch test: _____

Any current symptoms? (Yes, No) _____

If yes, list current symptoms: _____

☐ Suspicion of contact allergy associated with metal exposure

Type of metal exposure

- ☐ Cardiac implant (stent, pacemaker, etc.)
- ☐ Orthopedic implant (knee, hip or other)
- ☐ Gynecological implant or device
- ☐ Surgical hardware (plates, screws, wires, pins, rods, expanders, staples)
- ☐ Dental metal implant
- ☐ Dental metal appliance, prosthesis or filling
- ☐ Injection of aluminum containing vaccination
- ☐ Other _____

☐ Other reason Investigator believes the subject qualifies for study inclusion

- ☐ Past positive lymphocyte proliferation (or transformation) test to one of the dilution series metals being tested on this study

  Name of metal tested: _____

  Date of test: _____

  Results: _____
- ☐ Other _____

Figure 4: Type of Metal Exposure Questionnaire

Name of Procedure	_____	
Date of Procedure	_____	
Date symptoms first began	_____	
Patient's description of symptoms	_____	
Site(s) of onset	_____	
Symptoms currently present?	☐ Yes	☐ No
Duration	☐ Continuous	☐ Sporadic
If sporadic:		
How long do symptoms last?	_____	
What is the length of time between episodes?	_____	
Other symptoms? (itching, burning, pain, etc.)	_____	
Medication Taken?	☐ Yes	☐ No
If yes:		
Name of Medication	_____	
Start Date	_____	
Stop date (or ongoing)	_____	
Dose	_____	
Frequency	_____	
Route	_____	

Subjects who qualify for participation will have the investigational allergen panel(s) and reference allergens applied (with the exception of subjects enrolled in Germany who will not be tested with the reference allergens) to the paraspinal region of the upper back. Areas of contact dermatitis, uneven skin tones, tattoos, scars, moles, excessive hair or other skin disfiguration should be avoided. Upper arms may be used if there is not adequate space on the back for all panels. In summary, within these parameters, investigators will use their medical expertise to determine panel placement based on the features of each subject's back as they would normally do in clinical practice.

Panels may be further secured with medical tape (e.g., Scanpor® or patchProtect™). PatchMap may be used to document panel placement. The use of PatchMap will be required in Germany. The time of application will be documented.

The panel will be applied to the subject populations per the following:

- Sensitive subjects (those who have had a past positive patch test to at least one of the dilution series allergens) may be tested with the allergen panels(s) and corresponding reference allergen(s) (with the exception of subjects enrolled in Germany who will not be tested with the reference allergens) to which they have had a previous response.
  - The Investigator will use his or her experience and medical expertise to determine if a subject with a past positive patch result should be tested to all dilution series allergens or only to the allergen to which the subject has had the past response.
  - Subjects with a past positive response to copper, zinc, tin, manganese or molybdenum will be tested with the panel containing the past positive response allergen plus the other allergen(s) located on the same panel. Specifically, Panel 2 contains copper, zinc and tin, and Panel 3 contains manganese and molybdenum. Hence, subjects with a past positive response to one of the allergens on the panel will also be tested with the other allergen(s) located on the same panel. It will not be necessary to test the additional reference allergens.
- Subjects with suspicion of metal contact allergy potentially associated with a metal implant will be tested with all dilution series and reference allergens.
- All subjects will be tested with the excipient controls on Panel 6. The Investigator will use his or her experience and medical expertise to determine if a subject with a strong suspicion of metal contact allergy who has not undergone previous patch testing will benefit from being patch tested with the four common allergens (nickel, chromate, cobalt and gold) in order to provide the individual with a complete diagnosis of metal contact allergy. Should the Investigator decide that testing with the four common allergens will not be of benefit to the subject, the Investigator will cut Panel 6 as illustrated in Figure 9, (Section 11.1, Investigational Allergen Panels) before applying the panel.

10.2 Visit 2: Day 2

Prior to removal of the investigational allergen and reference panels, tape adhesion will be evaluated and scored according to the following adhesion 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

If adhesion is ‘Poor’ or ‘Detached’ the allergen sites that were not in contact with the skin at the time of panel removal will be identified.

Patches that do not remain in place (completely detached) for the intended wear period (approximately 48 hours or two days) will not be replaced. The subject will be asked to return for follow-up visits until all patch sites reactions have resolved but data from this subject will not be included in the analysis of positive responses necessary to determine optimal dose.

Subjects whose patches are not worn for the intended wear period may return to be retested after 3 weeks at the discretion of the Investigator providing the skin site remains free of conditions that may affect test results.

Factors such as oily skin, excess hair, wet panel, sweat/skin moisture, and activity-associated failure, which may potentially contribute to poor adhesion, will also be captured on the data collection form.

The patch panels will be removal and time documented. There will be at least a 15-minute wait between patch removal and the irritation evaluations. Following the 15-minute wait, tape sites (entire panel minus the chip sites) then the polyester chip sites will be evaluated for skin irritation.

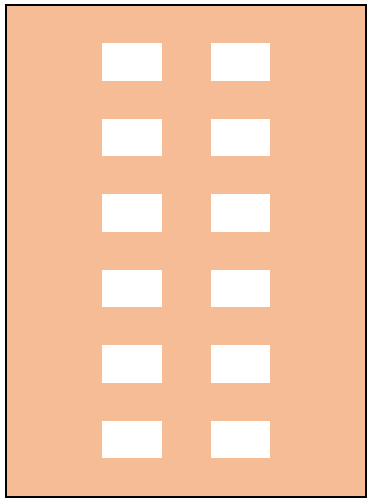
Skin irritation will be documented as follows:

- Panels 1-6: Irritation attributed to the adhesive used to adhere the panels to the skin (tape irritation) will be documented if the site of the entire panel with the exception of the chip sites, as shown below in Figure 5, shows signs of irritation
- Panels 1-6: Irritation attributed to the gel chips will be documented if all chip sites show signs of irritation. Note: To ensure that irritation resulting from skin contact with the polyester chip is not confused with an allergic response to a specific allergen, all of the polyester chip sites must present the same or nearly the same degree of irritation.
  - Panel 1: Chips on positions 1-4 and 7-10 will be evaluated for irritation
  - Panel 2: Chips on positions 1-12 will be evaluated for irritation
  - Panel 3: Chips on positions 1-4 and 7-10 will be evaluated for irritation
  - Panel 4: Chips on positions 1-12 will be evaluated for irritation
  - Panel 5: Chips on positions 1-4 and 7-10 will be evaluated for irritation
  - Panel 6: Chips on positions 1-4 and 7-9 will be evaluated for irritation. If Panel 6 is cut to avoid patch testing with the common allergens chips on the remaining positions will be evaluated.
- Panels 7-8: Irritation attributed to the adhesive used to adhere the panels to the skin (tape irritation) will be documented if the site of the entire panel with the exception of the patch test chambers show signs of irritation. This will be similar to the tape irritation example shown below in Figure 5 but the chambers used in the Finn Chamber product are round, not square.

Figure 5: Tape vs Chip Irritation Illustration

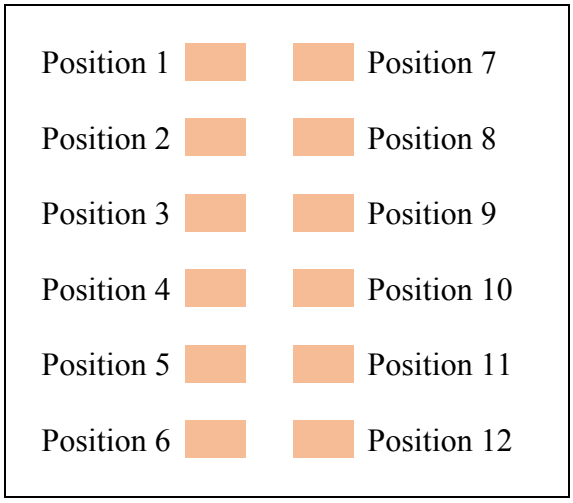
Tape Irritation:

Site of entire panel *with exception of chip sites* shows signs of skin irritation



Chip Irritation:

Only chip sites show signs of irritation.
NOTE: *All sites* must have the same or nearly the same degree of irritation.



Tape irritation will be evaluated according to the following score scale:

None:	No erythema or other sign of irritation
Weak:	Faint to definite pink erythema
Moderate:	Moderate erythema, definite redness
Strong:	Severe erythema, very intense redness

Polyester chip induced irritation will be evaluated according to the following score scale:

None:	No erythema or other signs of irritation
Weak:	Faint to definite pink erythema at **all** polyester chip sites*
Moderate:	Moderate erythema, definite redness at **all** polyester chip sites*
Strong:	Severe erythema, very intense redness at **all** polyester chip sites*

*To ensure that irritation resulting from skin contact with the polyester chip is not confused with an allergic response to a specific allergen, all of the polyester chip sites on each panel must present the same or nearly the same degree of irritation.

Subject self-report of itching and burning sensations for each allergen panel will be documented according to the following score scale:

None:	No discomfort
Weak:	Minimal discomfort
Moderate:	Definite discomfort
Strong:	Significantly bothersome; possible interference with sleep or daily activity

Subject-reported adverse events, worsening of a pre-existing condition/disease/symptom or changes to concomitant medications will be recorded.

10.3 Visit 3: Day 3-4

10.3.1 Tape and Polyester Chip Sites Irritation

Skin irritation due to the patch panel tape or chip will be evaluated and documented per the instructions presented in Section 10.2 Irritation that increases in severity or persists beyond visit 3 will be followed to resolution.

Tape Irritation will be evaluated according to the following score scale:

None:	No erythema or other sign of irritation
Weak:	Faint to definite pink erythema
Moderate:	Moderate erythema, definite redness
Strong:	Severe erythema, very intense redness

Polyester chip induced irritation will be evaluated according to the following score scale:

None:	No erythema or other signs of irritation
Weak:	Faint to definite pink erythema at **all** polyester chip sites*
Moderate:	Moderate erythema, definite redness at **all** polyester chip sites*
Strong:	Severe erythema, very intense redness at **all** polyester chip sites*

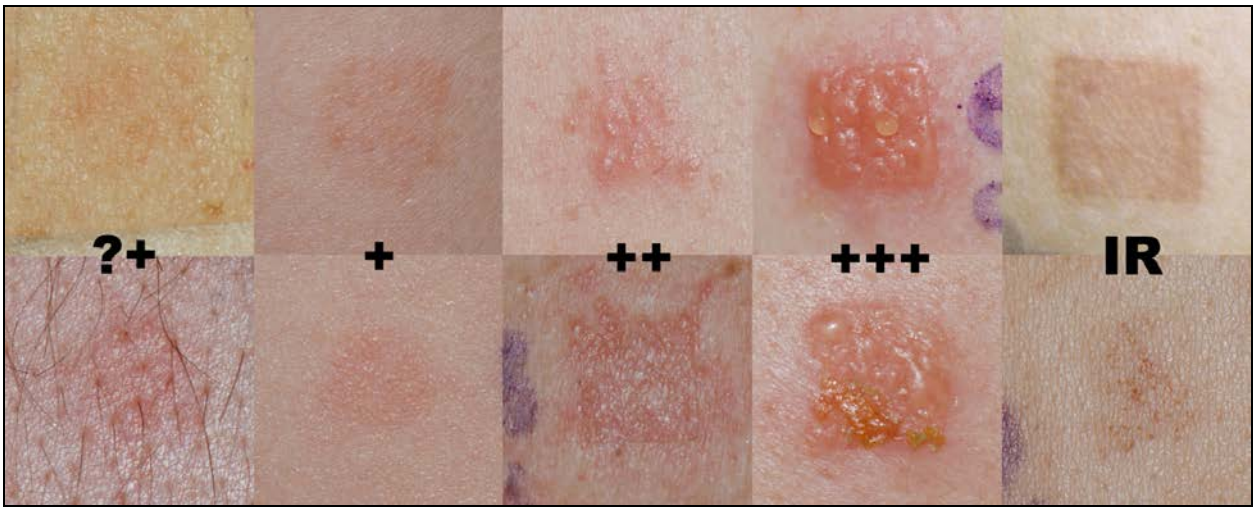
*To ensure that irritation resulting from skin contact with the polyester chip is not confused with an allergic response to a specific allergen, all of the polyester chip sites on each panel must present the same or nearly the same degree of irritation.

10.3.2 Allergic Skin Responses

Allergen site reactions will be evaluated for the presence of erythema, infiltration, papules, discrete vesicles and bullous reactions. These criteria will be used to score skin reactions according to standard patch testing guidelines established by the International Contact Dermatitis Research Group as discussed and shown below.

Skin reactions at test sites will be scored as: (Neg) negative; (IR) irritant reaction; (?+) doubtful reaction; (1+) weak positive reaction; (2+) strong positive reaction; or (3+) extreme positive reaction.

Figure 6: Reaction Criteria



Notation Description Interpretation

Neg	Negative, no skin changes in the tested area,
?+	Faint, non-palpable erythema, possibly few papules
+ (1+)	Palpable erythema, moderate edema or infiltrate, possibly few papules, no vesicles. Weak reaction.
++ (2+)	Strong infiltrate, numerous papules, possibly few vesicles present. Strong reaction.
+++ (3+)	Coalescing vesicles, bullae or ulceration. Extreme reaction.
IR	Inflammation sharply limited to the exposed area, lack of infiltrate, small petechiae, pustules, and efflorescences other than papules and vesicles.

Subject-reported adverse events, worsening of a pre-existing condition/disease/symptom or changes to concomitant medications will be recorded.

De-identified photographs of site reactions may be taken at the discretion of the Investigator and will not be reviewed or collected by the study sponsor.

10.4 Visit 4: Day 7-8 and Visit 5: Day 10-14

10.4.1 Tape and Polyester Chip Sites Irritation

Unresolved tape and or polyester chip induced irritation will be evaluated.

Tape irritation will be evaluated according to the following score scale:

None:	No erythema or other sign of irritation
Weak:	Faint to definite pink erythema
Moderate:	Moderate erythema, definite redness
Strong:	Severe erythema, very intense redness

Polyester chip induced irritation will be evaluated according to the following score scale:

None:	No erythema or other signs of irritation
Weak:	Faint to definite pink erythema at **all** polyester chip sites*
Moderate:	Moderate erythema, definite redness at **all** polyester chip sites*
Strong:	Severe erythema, very intense redness at **all** polyester chip sites*

Any response that is greater in severity than a strong irritation response may be graded as a potential allergic response as follows:

1+ Palpable erythema, moderate edema/ infiltrate, few papules, no vesicles. Weak reaction
2+ Strong infiltrate, numerous papules, possibly few vesicles present. Strong reaction
3+ Coalescing vesicles, bullae or ulceration. Extreme reaction

*To ensure that response to skin contact with the polyester chip is not confused with an allergic response to a specific allergen, all of the polyester chip sites per panel must present with the same or nearly the same response score.

10.4.2 Allergic Skin Responses

Allergen sites will be examined for the presence of erythema, infiltration, papules, discrete vesicles and bullous reactions and scored as follows: (-) negative, (1+) weak positive reaction, (2+) strong positive reaction, and (3+) extreme positive reaction.

All reactions (scores of 1+, 2+ or 3+) will be categorized as either late or persistent. Persistent reactions will be further clarified as either persistent-healing or persistent-escalating.

Definitions:

Late Reaction:	A reaction that initially appears at day 7-14 after application of panels.
Persistent Reaction:	A reaction that initially appears at day 2- 4 and persists through day 7-21 or beyond.
Persistent-Healing:	A persistent reaction that has not worsened since the previous evaluation, remains the same or appears to be healing.
Persistent-Escalating:	A persistent reaction that has worsened since the previous evaluation

Subject-reported adverse events, worsening of a pre-existing condition/disease/symptom or changes to concomitant medications will be recorded.

De-identified photographs of site reactions may be taken at the discretion of the Investigator and will not be reviewed or collected by the study sponsor.

10.5 Visit 6: Day 19-23

The investigative site will phone the subject to determine if there are any unresolved tape, polyester chip or allergen reactions at the test sites. If the subject reports no remaining reactions, the Investigator may choose at this point to complete the visit over the telephone.

- If the visit is conducted over the telephone all visit related paperwork including adverse events and/or changes to concomitant medications will be recorded.

Should the Investigator elect to see the subject in the office, the following procedures will be performed:

- Unresolved tape and or polyester chip induced irritation will be evaluated.
- Sites will be examined for the presence of erythema, infiltration, papules, discrete vesicles and bullous reactions and scored as follows: (-) negative, (1+) weak positive reaction, (2+) strong positive reaction, and (3+) extreme positive reaction.
- All reactions (scores of 1+, 2+ or 3+) will be categorized as either late or persistent. Late reactions are defined as reactions that initially occur at 7-10 days after application of the panels. Persistent reactions are defined as reactions that appear at day 2-4 and persist through day 7-21. Persistent reactions will be clarified as either persistent-healing or persistent-escalating. If a persistent-escalating reaction is noted at Visit 6, the investigator will determine required follow up action.
- Subject-reported adverse events, worsening of a pre-existing condition/disease/symptom or changes to concomitant medications will be recorded.
- De-identified photographs of site reactions may be taken at the discretion of the Investigator and will not be reviewed or collected by the study sponsor.

10.6 Post Visit 6

The Investigator will:

1. Evaluate tape or polyester chip induced irritation that increased in severity or persisted beyond visit 3. A potential allergic response may be considered if the skin response is consistent with criteria used to score 1+, 2+ or 3+ positive skin reactions according to standard patch testing guidelines established by the International Contact Dermatitis Research Group. The skin reaction must present with:
   - Palpable erythema, moderate edema or infiltrate, possibly few papules, no vesicles. Weak reaction (Score of 1+).
   - Strong infiltrate, numerous papules, possibly few vesicles present. Strong reaction (Score of 2+).
   - Coalescing vesicles, bullae or ulceration. Extreme reaction (Score of 3+).
2. Categorize the skin reactions at each site as either negative or positive. No skin reaction scores (1+, 2+ or 3+) assigned to during any of the reaction assessment visits, (Visits 3-6) would constitute a negative reaction. A positive skin reaction is defined as an assigned score of 1+, +2 or 3+ during at least one of the reaction assessment visits (Visits 3-6).

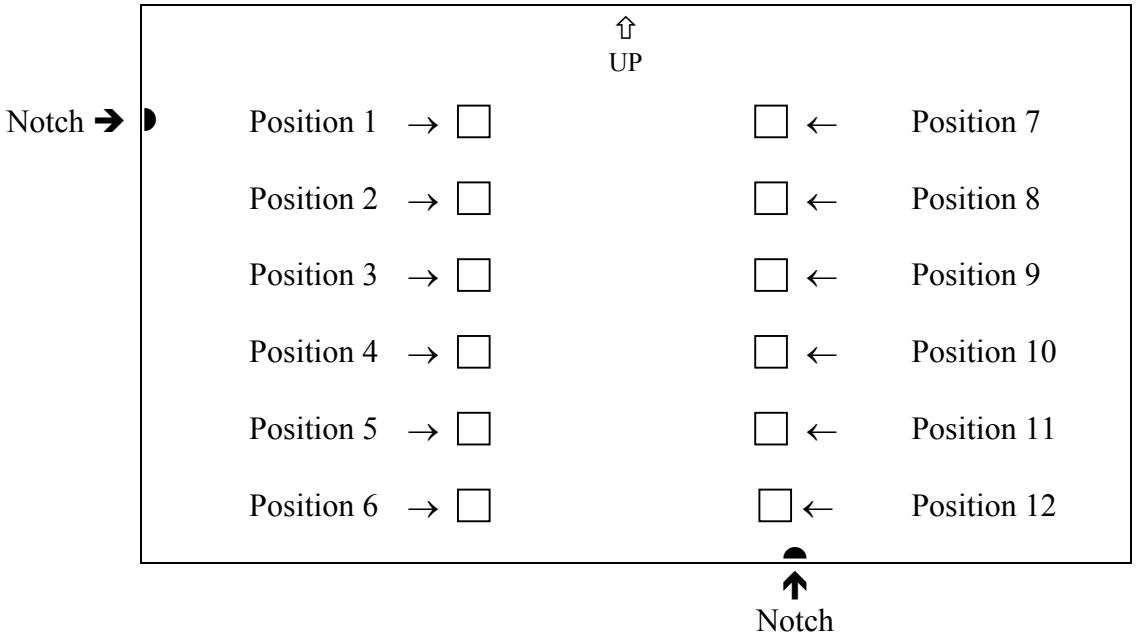
11.0 INVESTIGATONAL PRODUCT IDENTITY AND USE

11.1 Investigational Allergen Panels

Investigational T.R.U.E. TEST panels are produced, packed and labeled by the manufacturer SmartPractice Denmark, in Hillerød, Denmark. To protect tests against light and air, panels are sealed in aluminum foil opaque pouches. Panels are to be stored under refrigeration at 2-8^{o}C (36-46^{o}F). Products are labeled with allergen batch codes and expiration dating.

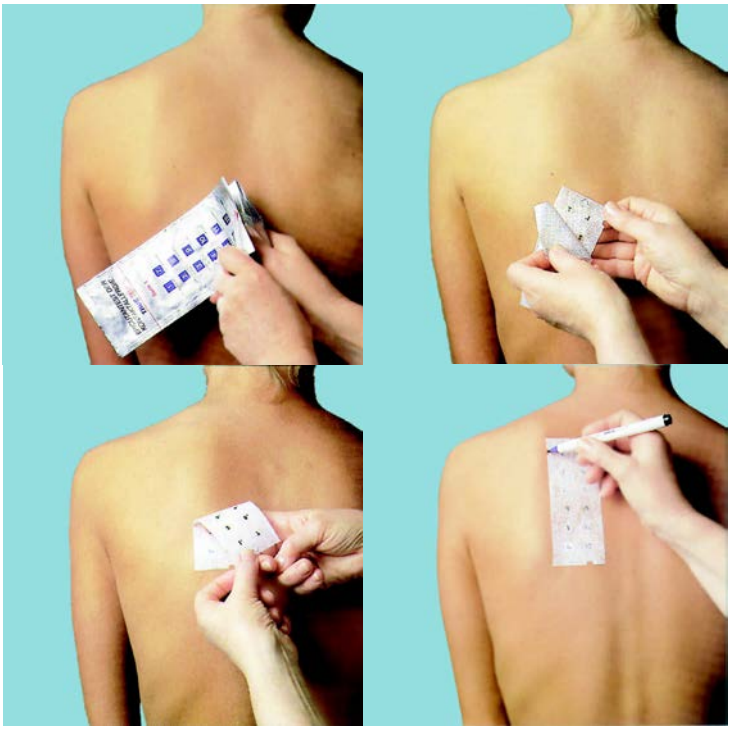
Each investigational panel consists of a 5.2 x 13.0 cm piece of surgical tape containing as many as 12 allergen patches. Although each allergen patch is assigned a ‘chip number’ for product identity, scoring will be by position placement as illustrated below.

Figure 7: Illustration of Position Numbering



An up arrow and two notches indicate the proper product orientation. The uppermost notch on the left side of the panel indicates the top left of the panel, the lower notch indicates the bottom of the panel. The patch chip numbering is unmistakable when the panel is removed from its pouch. Panels are to be applied with the numbers facing upright.

Figure 8: Application of Dilution Series Allergens



- Peel open package and remove test panel.
- Remove plastic covering.
- Position the panel to the paraspinal region of the upper back being careful to avoid areas of contact dermatitis, uneven skin tones, tattoos, scars, moles, excessive hair or other skin disfiguration.
- Ensure that each patch is in contact with the skin by smoothing the panel outward from the center
- Use a skin marker to indicate the location of the two notches on the panel
- Panel may be secured with additional tape, if needed.

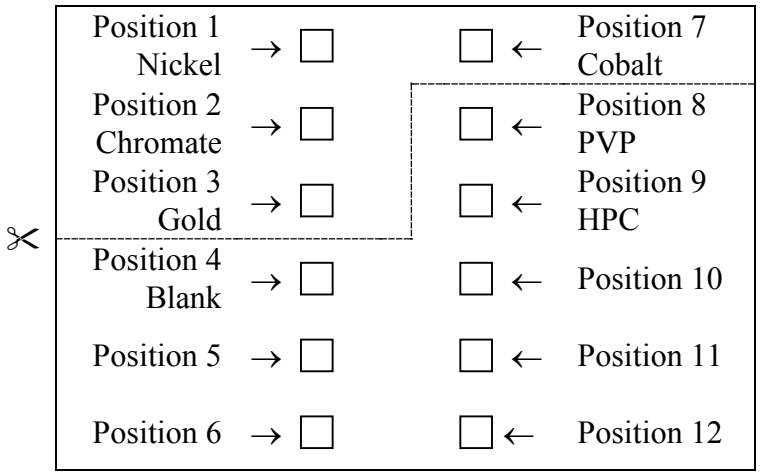
In order to differentiate between panels once they are applied the following chip numbers are used for each panel. The *set* of numbers used is unique to each panel so that individual panels can easily be identified:

	Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6
Chip numbers	1-4 and 7-10	13-24	25-28 and 31-34	25-36	3-6 and 9-12	1, 4, 5, 7, 8, 12, 28

The lead chemist in the department of pharmaceutical development at SmartPractice Denmark is responsible for the randomization of allergens on panels 1-5. The allergens will be randomized within each panel into three different configurations, per standard protocol. The different allergen configurations will be randomly assigned to subjects as they enter the study. The investigators and subjects will be aware of the allergens and the patch test doses on the test panels but will not be aware of the placement of the allergens on the individual panels (the different panel configurations). The key to the randomization scheme will be kept confidential at SmartPractice Denmark until it is forwarded to data management for analysis of the study results following completion of all study related procedures.

The allergens on panel 6 will not be randomized into different configurations. Should the Investigator elect to omit the common allergens from an individual's patch testing, the panel should be cut as shown below in Figure 9.

Figure 9: Panel 6 Configuration and Cutting Instructions



Unused portion of the panel is not to be discarded.

The remaining portion of the panel must be applied as it contains the excipient and blank patch controls. Scores for the blank (Position 4), PVP (Position 8), and HPC (Position 9) patches will be recorded in the case report forms as originally intended, in fields for positions 4, 8 and 9. Positions 1, 2, 3 and 7 will be documented in the case report form as 'not applied'.

All investigational products used in this study must be accounted for. Records must show amount and date of delivery of all test materials to the investigator. Likewise dates, amount, and signatures should document return of unused test panels. Product deliberately and/or accidentally destroyed by the investigator must also be documented.

The use of the investigational product for the individual subject, including number and identification of specific panels applied, application (or not) of common allergens and date and time of application and removal will be documented.

The following investigational panels with ascending doses of allergen and an excipient control will be used in this study.

Panel 1 Aluminum Dilution Series

Allergen	Excipients	Ascending Dosages (mg salt/cm²)	Randomized Among Positions
Aluminum chloride hexahydrate	Water, Ethanol (99.9%), PVP	0.040, 0.12, 0.36, 0.72	1-4 and 7-10
Aluminum lactate	Water, Ethanol (99.9%), PVP	0.047, 0.14, 0.42, 0.84	

Panel 2 Copper, Zinc and Tin Dilution Series

Allergen	Excipients	Ascending Dosages (mg salt/cm²)	Randomized Among Positions
Copper sulfate anhydrous	HPC, Water	0.013, 0.040, 0.080, 0.12	1, 2 and 7, 8
Zinc chloride	HPC, Water	0.013, 0.040, 0.080, 0.24	3, 4 and 9, 10
Tin chloride dihydrate	HPC, Water, Ethanol	0.018, 0.037, 0.11, 0.33	5, 6 and 11, 12

Panel 3 Manganese and Molybdenum Dilution Series

Allergen	Excipients	Ascending Dosages (mg salt/cm²)	Randomized Among Positions
Manganese chloride	HPC, Water	0.013, 0.040, 0.080, 0.24	1-4
Ammonium molybdate	HPC, Water	0.0067, 0.020, 0.040, 0.12	7-10

Panel 4 Titanium Dilution Series

Allergen	Excipients	Ascending Dosages (mg Ti/cm²)	Randomized Among Positions
Titanium peroxo citrate	PVP, Water	0.055, 0.11, 0.22	1-12
Titanium lactate	PVP, Ethanol	0.070, 0.14, 0.28	
Potassium titanium oxide oxalate	PVP, Ethanol	0.060, 0.12, 0.24	
Ammonium titanium oxide oxalate	PVP, Water	0.055, 0.11, 0.22	

Panel 5 Vanadium Dilution Series

Allergen	Excipient	Ascending Dosages (mg V/cm²)	Randomized Among Positions
Vanadium chloride	HPC, Water	0.0042, 0.0083, 0.025, 0.050	1-4 and 7-10
Vanadium oxide sulfate	HPC, Water	0.0042, 0.0083, 0.025, 0.050	

Panel 6 Common Allergens/Excipient Controls

Allergen/Control	Excipient	Dose (mg/cm²)	Position
Nickel sulfate	HPC	0.20	1
Potassium dichromate	PVP	0.054	2
Gold sodium thiosulfate	HPC	0.075	3
Blank Patch Negative Control	None	NA	4
Cobalt dichloride	HPC	0.020	7
PVP Negative Control	PVP	NA	8
HPC Negative Control	HPC	NA	9

The dose per unit area of experimental T.R.U.E. Test allergens is calculated based on the molecular weight of the compound. Exceptions are Titanium and Vanadium allergens as molecular weights are not known for all of these, hence the dose per unit area for Titanium and Vanadium allergens is based only on the metal part of the compound.

11.2 Reference Allergens

Commercially available reference allergens may be used or the reference allergen syringes may be prepared and labeled by the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden. The reference allergens will be stored in individual syringes under refrigeration at 2 - 8°C.

The Finn Chamber, polypropylene-coated, supplied by SmartPractice, will be used to apply the reference allergens. Reference allergens will be dispensed at the rate of 20 µl/chamber using a precision allergen dispenser.

Figure 10: Reference Allergen Preparation

Petrolatum allergens

⬅ Apply a ribbon of substance across the diagonal of chamber

Petrolatum allergens may be dispensed 24 hours prior to application

Liquid allergens

⬅ Add filter paper to chamber

Dispense liquid allergen to chamber ➡

Liquid allergens may not be dispensed
until immediately prior to patch application

Instructions for precision allergen dispenser:

1. Fill metering chamber with allergen using syringe plunger.
2. Wipe excess from tip.
3. Dispense allergen to chamber by depressing precision allergen dispenser plunger.

- The Aluminum Dilution Series will be tested concurrently with
  - Aluminum chloride hexahydrate, 10 % w/w in petrolatum
  - Aluminum lactate, 12 % w/w in petrolatum
- The Copper Dilution Series will be tested concurrently with copper sulfate anhydrous, 2 % w/w in petrolatum
- The Manganese Dilution Series will be tested concurrently with manganese chloride tetrahydrate, 2 % w/w in petrolatum
- The Molybdenum Dilution Series will be tested concurrently with ammonium molybdate, 1% aqueous solution.
- The Tin Dilution Series will be tested concurrently with tin chloride dihydrate, 1% w/w in petrolatum
- The Titanium Dilution Series will be tested concurrently with
  - Ammonium titanium peroxo citrate, 17% w/w in petrolatum
  - Ammonium titanium lactate, 34% aqueous solution
  - Potassium titanium oxide oxalate, 22% w/w in petrolatum
  - Ammonium Titanium oxide oxalate 19% w/w in petrolatum
- The Vanadium Dilution Series will be tested concurrently with
  - Vanadium chloride, 1% w/w in petrolatum
  - Vanadium oxide sulfate, 1.5% w/w in petrolatum
- The Zinc Dilution Series will be tested concurrently with zinc chloride, 2% w/w in petrolatum

NOTES: 1) The common allergens (Panel 6) will not be tested against reference allergens. 2) Subjects enrolled in Germany will not be tested with the reference allergens

12.0 DATA ANAYLSIS

12.1 Descriptive Statistics and Analysis

True disease status (allergic contact dermatitis to a specific allergen) is rarely definitively known. Patch test results, medical history, symptoms and provocation testing (if performed) are used to clinically diagnose disease status, and it is not uncommon to observe discordance among these diagnostic parameters. Moreover, symptoms and test results can change over time – presumably due to skin condition, decreased exposure or overall health. These factors contribute to the difficulty of assigning a positive disease status, i.e., sensitive status, to patients. In this clinical trial, subjects will be considered sensitive if they've had a past positive response even though they may not react to the reference allergen at the time of the study.

The frequency of ranked skin responses; positive (1+, 2+, 3+), negative, doubtful and irritant, will be calculated for each allergen dose (dilution series and reference) tested. The optimal allergen dose will be based on the following criteria:

- The lowest concentration of each dilution series allergen eliciting positive responses in a minimum of 15 subjects. Positive responses are defined as score of 1+, 2+ or 3+ during at least one reaction assessment visit. If a significant number of 3+ responses are elicited, the dose will be selected based on 1+ and 2+ responses.
- For all sites with the exception of Germany: Concordance will be measured using Cohen's kappa where less than 0% indicates no agreement, 0-20% indicates poor agreement, 20-40% indicates fair agreement, 40-60% indicates moderate agreement, 60-80% indicates good agreement and 80% or higher indicates very good agreement. Concordance will be measured using all subjects who are tested with each allergen and corresponding reference allergen.

Safety evaluations will be tabulated as follows:

- Frequency of tape and polyester chip induced irritation or allergic reactions at Visits 2 through 6 for the T.R.U.E. Test tape and polyester chips (Panels 1-6) and Finn Chamber tape (Reference allergen panels).
- Frequency of subject reported sensations of itching and/or burning for each allergen panel at patch removal.
- Frequency of positive (1+, 2+, 3+) skin reactions for each investigational and reference allergen dose at each post removal visit and overall.
- Frequency of negative, doubtful, irritant, late and persistent skin reactions for each investigational and reference allergen dose at each post removal visit (late and persistent reactions at visits 4, 5 and 6 only).
- Frequency of all adverse events. Documentation for all local and systemic adverse reactions classified by the investigator as possibly or definitely related to the study product (e.g., erythema, hyper-pigmentation, hypo-pigmentation, skin thinning or dermatitis flare) will include grade (mild, moderate or severe) and time point (clinic visit).

Statistical processing will be performed using SAS® software.

12.2 Sample Size and Power Calculations

A minimum of 15 subjects per dilution series allergen, who exhibit a positive skin response (score of 1+, +2 or 3+ during at least one reaction assessment visit) to the dilution series allergen and/or at least one of its corresponding reference allergens, is needed to complete the study.

Subjects with a past positive patch test response to at least one of the dilution series allergens will be tested with the allergen panel and corresponding reference allergen(s) to which they have had the previous response. Subjects with suspicion of metal contact allergy will be tested with all dilution series and reference allergens. All subjects will be tested with the excipient controls. The common allergens will be tested at the discretion of the investigator.

Past dose response study populations have included 20 adult subjects (per allergen) with a historical positive patch test to the corresponding reference allergen. In these studies, determination of the optimal test allergen dose was the lowest concentration eliciting a 1+, 2+ or 3+ positive reaction in 70-90% of subjects with the fewest number of 3+ reactions; therefore a minimum of 14 subjects with positive reactions was needed to determine optimal test allergen dose.

Due to the fact that not all of the metals being tested on this study have a large database of patients with past patch-test positive reactions, the inclusion criteria was modified to include subjects with a suspicion of metal allergy, in addition to those with a historical positive patch test. Because it is anticipated that not all subjects will test positive, the study will conclude when a total of 400 subjects have been tested.

The option of further testing may be considered for any allergen that elicits a minimum of 8 positive responses. In such a case, the protocol will be amended

12.3 Data Monitoring and Quality Assurance

The study will be performed according to ICH harmonized Tripartite Guidelines for Good Clinical Practice, national legislation and SmartPractice internal SOPs for Clinical Studies. Study sites will be visited at least once prior to initiation of the study, at approximately 8-12 week intervals or as needed during the study and at the conclusion of the study. The sponsor will perform all monitoring.

Subject data will be recorded in data collection forms. These data will be entered into SAS, for subsequent analysis, and proofread for entry errors. Statistical analysis will be performed according to Good Clinical Practice and ICH guidelines using SAS software per the respective analytical parameters.

13.0 ADVERSE EVENTS

13.1 Adverse Event Definitions

- **Adverse Event (AE):** Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related
- **Adverse Reaction or Adverse Drug Reaction (ADR):** Any adverse event caused by a drug.
- **Life-threatening Adverse Event or Life-threatening Suspected Adverse Reaction:** An adverse event or suspected adverse reaction is considered 'life-threatening' if, in the view of either the investigator or the sponsor, its occurrence places the subject at immediate risk of death. This does not include an adverse event or suspected adverse reaction that had it occurred in a more severe form, might have caused death.
- **Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction (SSAR):** An AE or SAR is considered serious if, in the view of either the investigator or sponsor it results in any of the following outcomes:
  - Death
  - A life-threatening adverse event
  - Inpatient hospitalization or the prolongation of existing hospitalization
  - A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
  - Is a congenital anomaly/birth defect

  Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
- **Suspected Adverse Reaction (SAR):** Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. 'Suspected adverse reaction' implies a lesser degree of certainty about causality than adverse reaction.
- **Suspected Unexpected Serious Adverse Reactions (SUSAR)**
  An adverse reaction that is both unexpected (not consistent with the applicable product information) and also meets the definition of a Serious Adverse Event/Reaction.
- **Unexpected Adverse Event (UAE)** or **Unexpected Suspected Adverse Reaction (USAR):**
  An AE or SAR is considered 'unexpected' if it is
  - not listed in the investigator brochure
  - not listed at the observed specificity
  - not consistent with the risk information described in the general investigational plan or in the current application.

13.2 Adverse Event Reporting Period

The adverse event-reporting period for each study subject begins at panel application and ends with the Day 21 visit. All adverse events that occur in study subjects during the adverse event-

reporting period specified in the protocol must be reported. In addition, any known adverse event that occurs in a study subject after Day 21 that the investigator determines as possibly related to the investigational product must also be reported as an adverse event.

13.3 Serious Adverse Events

Serious adverse events must be documented and reported to the sponsor, SmartPractice, within 24 hours of the investigator's learning/awareness of the event. This initial report must be followed by submission of more detailed adverse event within 5 working days.

Serious, unexpected reactions that are not fatal or life-threatening will be filed no later than 15 calendar days after first knowledge by the sponsor.

Determination of expectedness will be based on the contents of the Investigators Brochure.

13.4 Suspected Unexpected Serious Adverse Reactions (SUSARs)

An adverse event will be reported as a **SUSAR if the following three criteria** are met:

1. The event is serious, that is to say irrespective of the dose the event:
   - is fatal, and/or
   - is life-threatening for the subject, and/or;
   - makes hospital admission or an extension of the admission necessary, and/or
   - causes persistent or significant invalidity or work disability, and/or
   - manifests itself in a congenital abnormality or malformation
2. There is a certain degree of probability that the event is harmful, and an undesirable reaction to the experimental product being research, regardless of the administered dosage. In other words**,** there is an adverse reaction.
3. The adverse reaction is unexpected**.** That is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the Investigator's Brochure**.**

Fatal or life-threatening unexpected ADRs will be reported to all regulatory agencies and IRB/Ethics Committees by telephone, facsimile or in writing as soon as possible but no later than 7 calendar days after first knowledge by the sponsor followed by as complete a report as possible within 8 additional calendar days.

Deciding whether the adverse event meets the definition of a suspected adverse reaction is usually the most difficult determination. The sponsor will evaluate the available information and decide whether there is a reasonable possibility that the drug caused the adverse event and, therefore, that the event meets the definition of a suspected adverse reaction. The suspected adverse reaction must then be reported expeditiously in a safety report if it also meets the definitions of serious and unexpected.

The only response to a dermatological patch product that would trigger a SUSAR report would be anaphylactic reaction. There may be other reactions which would be categorized as SUSARs that are unknown at this time.

13.5 Severity

The investigator will provide an assessment of the severity of each adverse event by recording a severity rating on the appropriate AE reporting page of the subject's data collection form. (Severity, which is a description of the intensity of manifestation of the AE, is distinct from seriousness, which implies a subject outcome or AE-required treatment measure associated with a threat to life or functionality.) Severity will be assessed according to the following scale:

Mild:	Minimal symptoms / annoying / minimal discomfort. Does not interfere with subject's usual function
Moderate:	Definite discomfort / requires medication for relief. Interferes to some extent with subject's usual function
Severe:	Symptoms interfere significantly with subject's usual function (daily activity / sleep).

The Investigator must supervise topical treatment of subject dermatitis and patch test responses.

13.6 Relationship

The investigator will provide an assessment of causal relationship to the study product. The causality assessment must be recorded on the appropriate AE reporting page of the subject's data collection form. Relationship will be classified according to the following criteria:

Not Related	No association to the test drug. Related to other etiologies such as concomitant medications or conditions or subject's known clinical state. (An unrelated illness, including the worsening of a pre-existing illness, injury or accident).
Possibly Related	Uncertain association. Other etiologies are also possible.
Definitely Related	Clear-cut association with improvement upon withdrawal of the test drug. Not reasonably explained by the subject's known clinical state but not an anticipated event.

Adverse events that are to be reported as having a relationship with the study patch test panels include but are not limited to:

- A severe reaction to the test tape adhesive or allergen patch that either causes the subject to remove the panels prior to the 48 hour return visit or is determined by the investigator to be significantly greater in severity than expected or as described in the Investigator's Brochure.
- Any persistent, escalating, positive patch test reaction at the test site lasting beyond the day 21 evaluation that the investigator determines is severe.

- Any characteristic associated with a late or persistent reaction (i.e. hyper-pigmentation, hypo-pigmentation, pruritus or other) at the test site lasting beyond the day 21 evaluation that the investigator determines is severe.
  - **Hyperpigmentation:** The darkening of an area of the skin caused by an excess of pigment in a tissue as opposed to an erythematic response.
  - **Hypopigmentation:** The loss of skin color caused by melanocyte or melanin depletion, or a decrease in the amino acid tyrosine, which is used by melanocytes to make melanin.
  - **Pruritus:** Itching
- Any of the following reactions:
  - **Active Sensitization**: A positive reaction initially appearing at approximately 21 days after application. Sensitization may be confirmed if a positive reaction were to appear at 3-4 days after a repeated application of the allergen at a naïve test site.
  - **Acute Anaphylactic Reaction:** A severe whole body reaction to a chemical**.** Symptoms develop rapidly, often within seconds or minutes and may include, abdominal pain or cramping, abnormal (high pitched) breathing sounds, anxiety, confusion, cough, diarrhea, difficulty breathing, difficulty swallowing, fainting, lightheadedness, dizziness, hives, itchiness, nasal congestion, nausea, vomiting, palpitations, skin redness, slurred speech and wheezing. Patch test substances reported to cause immediate contact reactions are usually proteins, protein complexes (e.g., milk, latex) or medicinal substances (e.g., aminophenazone, ampicillin, bacitracin, neomycin, and bufexamac). Immediate reactions to a few cosmetic ingredients (balsam of Peru, paraben mix and fragrance mix) and miscellaneous items (epoxy resin and Formaldehyde) have also been reported but are very uncommon.
    Investigator may refer to the Guideline for Acute Therapy and Management of Anaphylaxis by Ring, et. al for more information
  - **Bacterial or Viral Infections:** These adverse reactions are considered exceedingly rare but have been reported.
  - **Ectopic Flare:** A positive patch test reaction accompanied by a flare of an existing or pre-existing dermatitis that was caused by the test allergen.
  - **Excited Skin Syndrome (Angry Back):** A state of skin hyper-reactivity induced by dermatitis on other parts of the body or by a strong positive skin-test reaction in which other patch test sites become reactive, especially to marginal irritants.
  - **Immediate Contact Urticaria (ICU):** An immediate but transient, localized, swelling and redness that occurs on the skin after direct contact with an offending substance. (Contact urticaria should be distinguished from allergic contact dermatitis where a dermatitis reaction develops hours to days after contact with the offending agent). Most non-allergic reactions remain local while ICU may produce generalized urticarial lesions. ICU reactions, reported in conjunction with patch testing, are due to higher allergen concentrations (e.g. 5% pet).
  - **Koebner Phenomenon:** Psoriasis plaques that form at the site of a skin injury. A positive patch test reaction in a subject with active psoriasis or lichen planus may reproduce these dermatoses at the patch site during the weeks after testing. Symptoms are cleared with topical corticosteroids.

- **Necrosis, Scarring and Keloids:** Testing with strong irritants (acids, alkalis or chemicals of unknown composition) may produce such adverse events but are considered extremely rare.
- **Pressure Effect:** An edematous area, typically most intense at the margins resulting from the application of patch tests. Dermatographic individuals are more likely to develop this reaction.
- **Tape Reaction:** Severe reaction to the tape or adhesive used to hold the patches in place such that the subject is forced to remove the patch prior to the 48 hour wear time.

13.7 Outcome and Follow-up

All unrelated adverse events should be followed until they are resolved or the subject's participation in the study ends. All serious, possibly related and definitely related adverse events should be followed until they resolve or until the investigator assesses them as chronic or stable even after the subject's participation in the study is over. Follow up of adverse events must be documented on the subject's data collection form.

SmartPractice will review all unexpected serious adverse events to determine if additional risk information should be added to the protocol and/or informed consent form. If it is determined to be necessary, the protocol and informed consent form will be revised and submitted according to regulatory requirements.

SmartPractice is required to notify each participating investigator informed of new information regarding the investigational drug, particularly with respect to adverse effects and safe use.

14.0 STUDY DISCONTINUATION

SmartPractice reserves the right to discontinue the study prior to inclusion of the intended number of subjects, but intends only to exercise this right for valid scientific or administrative reasons.

In the event that the study is prematurely terminated or interrupted all regulatory agencies will be notified within 15 days.

14.1 Criteria for Discontinuation

If a serious adverse event occurs that is considered associated with an investigational allergen or other patch test material, the study will immediately stop entering subjects until the case has been reviewed and further action and/or consequences, such as discontinuation of the study, will be considered.

If more than 20% of subjects experience an unacceptable adverse event associated with the investigational allergen or other patch test material, the cases will be reviewed and further action/consequences, i.e., discontinuation of the study, will be considered.

If more than one subject develops an overreaction, the study will immediately stop entering subjects until the case has been reviewed and further action and/or consequences, such as discontinuation of the study, will be considered.

The study may also be discontinued for the following reasons:
- Scientific reasons such as invalid study design, inappropriate test methods or subject inclusion.
- Inability to recruit and enroll sufficient subjects within the study period.
- Internal administrative reasons.

After discontinuation of the study, the investigator must collect all unused study products and other study material and complete appropriate data collection forms. The participating subjects should be informed and seen for a final visit.

15.0 DATA AND RECORD KEEPING

15.1 Data Collection Form

A completed data collection form is required for each subject included in the study. These completed original data collection forms are the sole property of SmartPractice. Data collection forms should not be made available in any form to third parties without written permission from SmartPractice, except to authorized representatives from regulatory authorities, Data Protection Agency and IEC.

15.2 Record Retention

To enable evaluations and/or audits from Health Authorities and/or SmartPractice, the Investigator agrees to keep appropriate records, including original Informed Consent/Assent forms, the identification of all participating subjects (sufficient information to link records, e.g., collection forms and hospital records) and supporting study documentation. Records will be stored, at the investigative site, as written in the ICH guideline section 4.9.5 until 2 years after the last approval of a marketing application in ICH region or at least 2 years have elapsed since the

formal discontinuation of clinical development of the investigational product. Storage of all original data collection forms and detailed records of the investigational product will be maintained by SmartPractice or their designated agent according to the current international clinical research regulations.

16.0 FINANCING AND INSURANCE

SmartPractice will supply all investigational panels, patch test chambers and reference allergens free of charge to physician-investigators.

Local physician-investigators will also be appropriately compensated for subject evaluation and management fees (E/M fees) that would otherwise not be reimbursable through health insurance. Study subjects will be nominally compensated per local and institutional clinical research guidelines. All compensation (subjects and investigators) will be subject to institutional, IRB/IEC, local and federal regulations and stipulations, and may therefore vary by study site. For clinical trials conducted in the U.S., SmartPractice assumes responsibility and is covered by separate Clinical Trials Liability Insurance (Travelers Property Casualty Co. of America, P # ZPP21N3040615I2); $1,000,000 per occurrence and $3,000,000 general aggregate.

17.0 DATA PUBLICATION

After study closing and/or completion, data analysis will be performed by SmartPractice or a designated agent. The results will be presented to the investigator(s). Based on these data a Clinical Study Report will be prepared in co-operation with the investigator(s). Clinical Study Report will be submitted to the regulatory authorities and/or any other regulatory agency that requires this information.

Any proposed oral or written use of study results by local and/or principle investigators must be submitted to SmartPractice for review at least 30 days prior to submission for publication, presentation, or use. This condition is stated so that SmartPractice will be aware of all written and oral presentations of the data and does not imply any editorial review or restriction of the contents of the presentation or use.

18.0 AMENDMENT I

The following changes have been made to the protocol.

The address for Dr. Pigatto	**has been changed from:**	**To:**
Section 2.0, Page 3, and Section 4.0, Page 8	University of Milano Dipartimento di Scienze Biomediche, Chirurgiche ed Odontostomatologiche H Galeazzi Via Galeazzi 4	University of Milano-Dipartimento di Scienze Biomediche, Chirurgiche ed Odontostomatologiche Servizio di Dermatologia/

	20161 Milano, Italy Tel: +39 02.33.14.139 e-mail: pigatto@pigattobersani.it	Servizio di dermatologia allergologica IRCCS Istituto Ortopedico Galeazzi Via R. Galeazzi 4 20161 Milano, Italy Tel: +39 0266.214761 e-mail: paolo.pigatto@unimi.it
The site for Dr. Pigatto Section 4.4, Page 10	**has been changed from:** University of Milano Dipartimento di Scienze Biomediche, Chirurgiche ed Odontostomatologiche H Galeazzi Via Galeazzi 4 20161 Milano, Italy	**To:** University of Milano- Dipartimento di Scienze Biomediche, Chirurgiche ed Odontostomatologiche Servizio di Dermatologia/ Servizio di dermatologia allergologica IRCCS Istituto Ortopedico Galeazzi Via R. Galeazzi 4 20161 Milano, Italy
Primary Endpoint Section 4.8, Page 12 Section 4.15, Page 19-20 Section 7.1, Page 27 and Section 12.1, Page 51	**has been changed from:** Approximately 50% of the positive responses attributed to the dilution series allergen;	**To:** At least 50% of the positive responses attributed to the dilution series allergen;
Secondary Endpoint Section 4.8, Page 12-13 Section 4.15, Pages 19-20 Section 7.1, Page 27-28 and Section 12.1, Page 51	**has been changed from:** Frequency of adverse events.	**To:** Frequency of all adverse events. Documentation for all local and systemic adverse reactions classified by the investigator as possibly or definitely related to the study product (e.g., erythema, hyper-pigmentation, hypo-pigmentation, skin thinning or dermatitis flare) will include grade (mild, moderate or severe) and time point (clinic visit).

Exclusion Criteria b Section 9.2.2, Page 31	**has been changed from:** Topical treatment with corticosteroids or other immunosuppressive agents on or near the test area during the 14 days prior to inclusion in this study.	**To:** Topical treatment with corticosteroids or other immunosuppressive agents on or near the test area 14 days prior to inclusion through the end of the subject's participation in the study.
Exclusion criteria c Section 9.2.2, Page 31	**has been changed from:** Systemic treatment with corticosteroids (equivalent to > 10 mg prednisone) or other immunosuppressive agents during the 14 days prior to inclusion in this study. Inhaled treatments are permitted. NOTE: Steroidal nose or eye drops are permitted.	**To:** Systemic treatment with corticosteroids (equivalent to > 10 mg prednisone) or other immunosuppressive agents 14 days prior to inclusion through the end of the subject's participation in the study. Inhaled treatments and steroidal nose or eye drops are permitted.
Exclusion Criteria d Section 9.2.2, Page 31	**has been changed from:** Treatment with ultraviolet (UV) light (including tanning) during the 3 weeks prior to inclusion in this study.	**To:** Treatment with ultraviolet (UV) light (including tanning) during the 3 weeks prior to inclusion through the end of the subject's participation in the study.
Exclusion Criteria f Section 9.2.2, Page 31	**has been added.** Known or suspected infection of the skin, joints or other site(s) associated with metal exposure	
Exclusion Criteria h Section 9.2.2, Page 31	**has been added.** A condition such as; psoriasis, dermatitis herpetiformis, mycosis fungoides or cutaneous T-cell lymphoma that may confound the evaluation of allergic contact dermatitis.	
The following paragraph Section 10.1, Page 34	**has been added.** Age of diagnosis, distribution, severity and current medication use (including those being withheld for the duration of the study) will be captured for subjects who exhibit concurrent atopic dermatitis and irritant dermatitis	

The following paragraphs	**have been added**
Section 10.2, Pages 37-38	Patches that do not remain in place (completely detached) for the intended wear period (approximately 48 hours or two days) will not be replaced. The subject will be asked to return for follow-up visits until all patch sites reactions have resolved but data from this subject will not be included in the analysis of positive responses necessary to determine optimal dose. Subjects whose patches are not worn for the intended wear period may return to be retested after 3 weeks at the discretion of the Investigator providing the skin site remains free of conditions that may affect test results

The following paragraph	**has been changed from:**	**To:**
Section 11.1, Pages 46	Allergens on panels 1-5 will be randomized within each panel into three different configurations, per standard protocol. These different allergen configurations will be randomly assigned to subjects as they enter the study. The investigators and subjects will be aware of the allergens and the patch test doses on the test panels but will not be aware of the placement of the allergens on the individual panels (the different panel configurations).	The lead chemist in the department of pharmaceutical development at SmartPractice Denmark is responsible for the randomization of allergens on panels 1-5. The allergens will be randomized within each panel into three different configurations, per standard protocol. The different allergen configurations will be randomly assigned to subjects as they enter the study. The investigators and subjects will be aware of the allergens and the patch test doses on the test panels but will not be aware of the placement of the allergens on the individual panels (the different panel configurations). The key to the randomization scheme will be kept confidential at SmartPractice Denmark until it is forwarded to data management for analysis of the study results following completion of all study related procedures.

19.0 AMENDMENT II

The following changes have been made to the protocol.

The credentials in:	have been changed from:	To:
Section 2.0, Page 3 and Section 4.3, Page 8	Thomas Rustemeyer, MD, PhD	Prof. dr. Thomas Rustemeyer
The phone number in:	**has been removed.**	
Section 4.3	or +31.20.670.4230	
The wording in:	**has been changed from:**	**To:**
Section 4.9, Page 13 Section 9.0, Page 30 Section 9.1, Page 30 Section 10.1, Page 37 (bullet 3) and Section 12.2, Page 52	All subjects will be tested with the common allergen/excipient control panel.	All subjects will be tested with the excipient controls. The common allergens will be tested at the discretion of the investigator.
The wording in:	**has been changed from:**	**To:**
Section 6.12, Page 26	Metal Panel T.R.U.E. TEST will be indicated for patients in whom the physician suspects metal exposure has resulted in: ▪ Cardiac implant (stent, pacemaker, etc.) ▪ Orthopedic implant (knee, hip or other) ▪ Gynecological implant or device ▪ Surgical hardware (plates, screws, wires, pins, rods, expanders, staples) ▪ Dental metal implant ▪ Dental metal appliance, prosthesis or filling ▪ Inflammation associated with an oral metal implant: (no changes to remainder of section)	Metal Panel T.R.U.E. TEST will be indicated for patients exposed to cardiac implants (stent, pacemaker, etc.), orthopedic implants (knee, hip or other), gynecological implants or devices, surgical hardware (plates, screws, wires, pins, rods, expanders, staples), dental metal implants, or dental metal appliances, prosthesis or fillings whose exposure to has resulted in: ▪ Inflammation associated with an oral metal implant: (no changes to remainder of section)

The wording in: Section 9.2.1, c, Page 30	**has been added.** 1) systemic birth control (i.e., oral contraceptives, skin patch, vaginal ring, implant, injection, or intrauterine device (IUD), which contains either a hormone or copper); 2) double barrier method (i.e., diaphragm, cervical cap, sponge, condom with spermicide);.	
The note in: Section 9.2.1, Page 31	**has been added.** (Note: Cervical cap and abstinence from sexual intercourse will not be considered as acceptable methods of contraception for subjects enrolled in Japan)	
The wording in: Section 9.2.2, a, Page 31	**has been added.** Breastfeeding may be resumed upon completion of the study.	
The visit schedule in: Section 10.0, Pages 33	**has been added.**	
The note in: Section 10.1, bullet 1, Page 34	**has been added.** (Note: Legal representative in addition to subject must sign consent form for subjects aged 18-19, enrolled in Japan.)	
The wording in: Section 10.1, Page 34	**has been changed from:** Urine pregnancy test (if needed)	**To:** Dipstick urine pregnancy test for all females of childbearing potential (mouse anti-beta hCG antibody conjugated to colloidal gold and goat anti-alpha hCG antibody coated on the membrane)
The wording in: Section 10.3.2, Page 41	**has been changed from:** Skin reactions at test sites will be scored as: (Neg) negative; (IR) irritant reaction; (?+) doubtful reaction; (+) weak positive reaction; (+) strong positive reaction; or (+) extreme positive reaction.	**To:** Skin reactions at test sites will be scored as: (Neg) negative; (IR) irritant reaction; (?+) doubtful reaction; (1+) weak positive reaction; (2+) strong positive reaction; or (3+) extreme positive reaction.

The wording in:	**has been changed from:**	**To:**
Section 10.4.2, Pages 42-43	All reactions (scores of 1+, 2+ or 3+) will be categorized as either late or persistent and characterized by the presence of hyper/hypo pigmentation and/or pruritus or other described symptom. Late reactions are defined as reactions that initially occur at 7-10 days after application of the panels. Persistent reactions are defined as reactions that appear at day 2- 4 and persist through day 7-21. Persistent reactions will be clarified as either persistent-healing or persistent-escalating.	All reactions (scores of 1+, 2+ or 3+) will be categorized as either late or persistent and characterized by the presence of hyper/hypo pigmentation and/or pruritus or other described symptom. Persistent reactions will be further clarified as either persistent-healing or persistent-escalating. Definitions: Late Reaction: A reaction that initially appears at day 10-14 after application of panels. Persistent Reaction: A reaction that initially appears at day 2- 4 and persists through day 7-21 or beyond. Persistent-Healing: A persistent reaction that has not worsened since the previous evaluation, remains the same or appears to be healing. Persistent-Escalating: A persistent reaction that has worsened since the previous evaluation. Hyperpigmentation: The darkening of an area of the skin caused by an excess of pigment in a tissue as opposed to an erythematic response. Hypopigmentation: The loss of skin color caused by melanocyte or melanin depletion, or a decrease in the

		amino acid tyrosine, which is used by melanocytes to make melanin. Pruritus: Itching
The wording in: Section 11.1, Page 44	**has been added.** Panels are to be stored under refrigeration at 2-8ºC (36-46ºF).	
The wording in: Section 11.1, Page 47	**has been added.** The allergens on panel 6 will not be randomized into different configurations. Should the Investigator elect to omit the common allergens from an individual's patch testing, the panel should be cut below positions 2 and 8 as shown in Figure 5.	
Figure 9, Panel 6 Configuration and Cutting Instructions in: Section 11.1, Page 47	**has been added.**	
The wording in Section 11.1, Page 47	**has been added.** Unused portion of the panel is not to be discarded.	
The wording in: Section 11.1, Page 47	**has been changed from:** The use of the investigational product for the individual subject, including the subject number, date of application of the test panel, and date of removal of the test panel will be documented.	**To:** The use of the investigational product for the individual subject, including number and identification of specific panels applied, application (or not) of common allergens and date and time of application and removal will be documented.
The Dilution Series Tables for Panels 1-5 in: Section 11.1, Pages 48-49	**have been changed from:** 3 columns headed: (Allergen) Dilution Series Excipient Dose	**To:** 4 columns headed: Allergen Excipients Ascending Dosages Randomized Among Positions
The Panel 6 Common Allergens/Excipient Control Table in: Section 11.1, Page 49	**has been changed from:** 3 columns headed: Allergen/Control, Excipient,	**To:** 4 columns headed: Allergen/Control, Excipient,

	Dose	Dose, Position
The wording in: Section 16.0, Page 59	**has been changed from:** For clinical trials conducted in the U.S., SmartPractice assumes responsibility and is covered by separate Clinical Trials Liability Insurance (Columbia Casualty Company, Policy number: ADT2097453381); $1,000,000 per occurrence and $3,000,000 general aggregate.	**To:** For clinical trials conducted in the U.S., SmartPractice assumes responsibility and is covered by separate Clinical Trials Liability Insurance (Travelers Property Casualty Co. of America, P # ZPP21N3040615I2); $1,000,000 per occurrence and $3,000,000 general aggregate.

20.0 AMENDMENT III

The contact information for Kayoko Matsunaga, M.D., Ph.D. in: Section 1.0, Page 1	**has been changed from:** Vice-President of Fujita Health University Professor and Chairperson Department of Dermatology	**To:** Professor Department of Integrative Medical Science for Allergic Disease
The address for Akiko Yagami, MD, PhD in: Section 2.0, Page 4 Section 4.4, Page 9 and Section 4.4, Page 10	**has been changed from:** Associate Professor of Department of Dermatology Fujita Health University School of Medicine 1-98, Dengakugakubo, Kutsukake-cho, Toyake, Aichi 470-1192, Japan	**To:** Professor, Department of Allergology, Fujita Health University Second Educational Hospital 3-6-10, Otobashi, Nakagawa-ku, Nagoya, 454-8509, Japan
A new Investigator in: Section 2.0, Page 4 and Section 4.3, Page 9	**has been named.** Hiromi Kanto MD, PhD Department of Dermatology, School of Medicine Faculty of Medicine, Toho University Toho University Omori Medical Center 6-11-1, Omori-Nishi, Ota-ku Tokyo 143-8541 Japan	
The following Investigator Section 2	**has been removed.** **Risa Tamagawa-Mineoka MD**	

Section 4.3	Department of Dermatology Graduate School of Medical Science Kyoto Prefectural University of Medicine 465 Kajii cho, Kawaramachi Hirokoji, Kamigyo ku Kyoto 602-8566, Japan
A new Sub-Investigator in: Section 4.3, Page 9	**has been added.** Professor Yoshiaki Kubo Head of Dermatological Science, Tokushima University Hospital 2-50-1 Kuramoto-cho Tokushima City, Tokushima Japan 7708503
The site in: Section 4.4, Page 10	**has been added.** Department of Dermatology, School of Medicine Faculty of Medicine, Toho University Toho University Omori Medical Center 6-11-1, Omori-Nishi, Ota-ku, Tokyo 143-8541 Japan
The site in: Section 4.4	**has been removed.** Department of Dermatology Graduate School of Medical Science Kyoto Prefectural University of Medicine 465 Kajii cho, Kawaramachi Hirokoji, Kamigyo ku Kyoto 602-8566, Japan
The IRB in: Section 4.5, Page 11	**has been added.** Toho University Omori Medical Center Institutional Review Board Toho University Omori Medical Center 6-11-1, Omori-Nishi, Ota-ku Tokyo 143-8541 Japan
The IRB in: Section 4.5	**has been removed.** Ethical Review Board, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

The wording in:	**has been changed from:**	**To:**
Section 4.7, Page 12, and Section 8.1, Page 28	This is a prospective, multi-center, double-blind, randomized design	This is a prospective, multi-center, randomized, double-blind design.

The sentences in: Section 4.7, Page 12, and Section 8.1, Page 29	**have been added.** That is, the allergen doses on each panel will be randomized into three different configurations, which will be randomly

	assigned to subjects as they enter the study. Although the investigators and subjects will know which allergen is being tested, they will be blinded to the placement of the allergen doses within each panel.	
The primary endpoint in: Section 4.8, Page 12 Section 4.15,Page 19-20 Section 7.1 , Page 27, and Section 12.1, Page 51	**has been changed from:** Determination of optimal test allergen dose as: ▪ The lowest dilution series dose eliciting a positive response (defined as score of 1+, 2+ or 3+ during at least one reaction assessment visit) in a minimum of 15 subjects who respond positively to the dilution series allergen, associated reference allergen or both. If a significant number of 3+ reactions are elicited, the optimal allergen dose based on 1+ and 2+ positive reactions will be selected. ▪ At least 50% of the 15 positive responses attributed to a dilution series allergen. ▪ Moderate to very good concordance (measured using Cohen's kappa, includes all subjects who test each allergen) between the dilution series and reference allergens. [less than 0% no agreement, 0-20% poor, 20-40% fair, 40-60% moderate, 60-80% good, 80% or higher very good]. A positive response to at least one of the reference allergens associated with each dilution	**To:** Determination of optimal test allergen dose as: ▪ The lowest concentration of each dilution series allergen eliciting positive responses in a minimum of 15 subjects. Positive responses are defined as score of 1+, 2+ or 3+ during at least one reaction assessment visit. If a significant number of 3+ responses are elicited, the dose will be selected based on 1+ and 2+ responses. ▪ For all sites with the exception of Germany: Concordance will be measured using Cohen's kappa where less than 0% indicates no agreement, 0-20% indicates poor agreement, 20-40% indicates fair agreement, 40-60% indicates moderate agreement, 60-80% indicates good agreement and 80% or higher indicates very good agreement. Concordance will be measured using all subjects who are tested with each allergen and corresponding reference allergen.

	series (in the cases where there are two or more reference allergens) will be considered reference positive.	
The sentence in: Section 4.9, Page 13, and Section 9.0, Page 30	**has been added.** The study population should include a reasonable representation of patients who have undergone a metal replacement procedure.	
The paragraphs in: Section 4.10 Pages 13 and Section 12.2, Page 52	**have been changed from:** Because not all subjects will have a positive reaction, the total number of subjects tested will be greater than 15 per dilution series allergen. The study will conclude when a total of 400 subjects have been tested. However, further testing may be required on any of the dilution series allergens that elicit a minimum of 8 positive responses; in such a case, the protocol will be amended.	**To:** Past dose response study populations have included 20 adult subjects (per allergen) with a historical positive patch test to the corresponding reference allergen. In these studies, determination of the optimal test allergen dose was the lowest concentration eliciting a 1+, 2+ or 3+ positive reaction in 70-90% of subjects with the fewest number of 3+ reactions; therefore a minimum of 14 subjects with positive reactions was needed to determine optimal test allergen dose. Due to the fact that not all of the metals being tested on this study have a large database of patients with past patch-test positive reactions, the inclusion criteria was modified to include subjects with a suspicion of metal allergy, in addition to those with a historical positive patch test. Because it is anticipated that not all subjects will test positive, the study will conclude when a total of 400 subjects have been tested

		whether or not the 15 positive response subjects per allergen quota is met. The option of further testing may be considered for any allergen that elicits a minimum of 8 positive responses. In such a case, the protocol will be amended.
The sentence in: Section 4.12 , Page 16-17 and Section 11.2, Page 51	**has been added.** Subjects enrolled in Germany will not be tested with the reference allergens	
The sentences in: Section 4.13, Page 17, C, D and E, Section 10.3.2, Page 41 Section 10.4.2 , Page 43 and Section 10.5, Page 43	**have been changed from:** Photographs of site reactions may be taken at the discretion of the Investigator	**To:** De-identified photographs of site reactions may be taken at the discretion of the Investigator and will not be reviewed or collected by the study sponsor.
The sentence in: Section 4.14 (Visit 4), Page 18 Section 4.14 (Visit 5), Page 18-19	**has been changed from:** Categorization of positive reactions as late or persistent; characterized by the presence of hyper/hypo pigmentation and/or pruritus or other described symptoms	**To:** Categorization of positive reactions as late or persistent
The sentence in: Section 4.16 , Page 20 and Section 8.6, Page 29	**has been added.** When this maximum number of subjects has been reached the sponsor of the study will inform the investigator that the study has been concluded at that site.	
The sentence in: Section 4.16 , Page 20 and Section 8.6, Page 29	**has been changed from:** The final report will be submitted to the Principal Investigator within 120 days of data base lock.	**To:** The final report will be submitted to the Principal Investigator within 120 days of data base lock and to the FDA, European Commission and PMDA within 1 year of study completion.
The paragraph in: Section 8.3, Page 28-29	**has been changed from:** Independent IRB/Ethics	**To:** The study protocol, any

	committee approval of study protocol, any amendments and the informed consent form will be obtained prior to study initiation or prior to implementation of protocol changes. This study will not proceed without receipt of all written approvals.	amendments and the informed consent will receive favorable approvals from all regulatory authorities (US-FDA, Switzerland-Health Authority Swissmedic, Italy-Italian Medicines Agency, Netherlands-Medicines Evaluation Board, Germany-Paul Ehrlich Institut, Japan-PMDA) and from independent IRB/Ethics committees for each study site prior to study initiation or prior to implementation of protocol changes. This study will not proceed without receipt of all written approvals.
The sentence in: Section 9.0, Page 29	**has been changed from:** Subjects with a past positive patch test response to at least one of the dilution series allergens will be tested with the allergen panel and corresponding reference allergen(s) to which they have had the previous response.	**To:** Subjects with a past positive patch test response to at least one of the dilution series allergens will be tested with the allergen panel and corresponding reference allergen(s) (with the exception of subjects enrolled in Germany who will not be tested with the reference allergens) to which they have had the previous response.
The sentence in: Section 9.1, Page 30	**has been changed from:** Sensitive subjects (those who have had a past positive patch test to at least one of the dilution series allergens) will be tested with the allergen panels(s) and corresponding reference allergen to which they have had a previous response.	**To:** Sensitive subjects (those who have had a past positive patch test to at least one of the dilution series allergens) will be tested with the allergen panels(s) and corresponding reference allergen(s) (with the exception of subjects enrolled in Germany who will not be tested with the reference

		allergens) to which they have had a previous response.

The notes in:	**have been added.**
Section 9.2.1, c, Page 31 (bullets 2 and 3)	o A double-barrier method must be used for all subjects enrolled in Switzerland who are practicing non-systemic methods of birth control. o Abstinence from sexual intercourse will not be considered an acceptable method of contraception for subjects enrolled in Switzerland.
The following exclusion criteria required in Germany only have been added to:	
Section 9.2.2, Page 31-32	▪ Alcohol abuse as well as drug and/or medication abuse ▪ Severe psychiatric, psychological or neurological disorders ▪ Patients in any relationship or dependency with the sponsor and/or investigator ▪ General inflammatory as well severe acute and chronic inflammatory diseases ▪ Malignancy during the previous 5 years ▪ Completed or ongoing long-term treatment with tranquilizer or psycho active drug

The sentence in:	**has been changed from:**	**To:**
Section 9.3, Page 32	Data from withdrawn subjects may be used in safety analysis.	Data from withdrawn subjects may be used in the safety analysis and will remain anonymous.

The sentence in:	**has been added.**
Section 9.3, Page 32 (bullet 1)	Overreaction to an allergen. Defined as a response greater than Extreme Positive (3+). A severe reaction to the test tape adhesive or allergen patch that either causes the subject to remove the panels prior to the 48 hour return visit or is determined by the investigator to be significantly greater in severity than expected or as described in the Investigator's Brochure.
The sentence in:	**has been added.**
Section 9.4, Page 33	The Investigator may withdraw a subject if the subject does not meet the study requirements.

The sentence in:	**has been changed from:**	**To:**
Section 10.1, Page 36	Subjects who qualify for participation will have the investigational allergen panel(s) and reference	Subjects who qualify for participation will have the investigational allergen panel(s) and reference

	allergens applied to the paraspinal region of the upper back	allergens applied (with the exception of subjects enrolled in Germany who will not be tested with the reference allergens) to the paraspinal region of the upper back

The sentences in:	**have been added.**
Section 10.1, Page 36	PatchMap may be used to document panel placement. The use of PatchMap will be required in Germany.

The sentence in:	**has been added.**
Section 10.1, Page 36	In summary, within these parameters, investigators will use their medical expertise to determine panel placement based on the features of each subject's back as they would normally do in clinical practice.

The sentence in:	**has been changed from:**	**To:**
Section 10.1, Page 37	Sensitive subjects (those who have had a past positive patch test to at least one of the dilution series allergens) will be tested with the allergen panels(s) and corresponding reference allergen(s) to which they have had a previous response.	Sensitive subjects (those who have had a past positive patch test to at least one of the dilution series allergens) may be tested with the allergen panels(s) and corresponding reference allergen(s) (with the exception of subjects enrolled in Germany who will not be tested with the reference allergens) to which they have had a previous response.

Two bullets in:	**have been added.**
Section 10.1, Page 37	➢ The Investigator will use his or her experience and medical expertise to determine if a subject with a past positive patch result should be tested to all dilution series allergens or only to the allergen to which the subject has had the past response. ➢ Subjects with a past positive response to copper, zinc, tin, manganese or molybdenum will be tested with the panel containing the past positive response allergen plus the other allergen(s) located on the same panel. Specifically, Panel 2 contains copper, zinc and tin, and Panel 3 contains manganese and molybdenum. Hence, subjects with a past positive response to one of the allergens on the panel will also be tested with the other allergen(s) located on the same

panel. It will not be necessary to test the additional reference allergens.

The final bullet in: Section 10.1, Page 37	**has been changed from:** ▪ All subjects will be tested with the excipient controls on Panel 6. The common allergens will be tested at the discretion of the investigator.	**To:** ▪ All subjects will be tested with the excipient controls on Panel 6. The Investigator will use his or her experience and medical expertise to determine if a subject with a strong suspicion of metal contact allergy who has not undergone previous patch testing will benefit from being patch tested with the four common allergens (nickel, chromate, cobalt and gold) in order to provide the individual with a complete diagnosis of metal contact allergy. Should the Investigator decide that testing with the four common allergens will not be of benefit to the subject, the Investigator will cut Panel 6 below positions 2 and 8 as illustrated in Figure 9, (Section 11.1, Investigational Allergen Panels) before applying the panel.
The sentence in: Section 10.2, Page 38	**has been changed from:** Following the 15 minute wait, panel 1-6 tape sites (entire panel minus the chip sites) then the polyester chip sites will be evaluated for skin irritation.	**To:** Following the 15 minute wait, tape sites (entire panel minus the chip sites) then the polyester chip sites will be evaluated for skin irritation.
The section on skin irritation	**has been added.**	

documentation:

Section 10.2, Page 38

Skin irritation will be documented as follows:

- Panels 1-6: Irritation attributed to the adhesive used to adhere the panels to the skin (tape irritation) will be documented if the site of the entire panel with the exception of the chip sites, as shown below in Figure 5, shows signs of irritation
- Panels 1-6: Irritation attributed to the gel chips will be documented if all chip sites show signs of irritation. Note: To ensure that irritation resulting from skin contact with the polyester chip is not confused with an allergic response to a specific allergen, all of the polyester chip sites must present the same or nearly the same degree of irritation.
  - Panel 1: Chips on positions 1-4 and 7-10 will be evaluated for irritation
  - Panel 2: Chips on positions 1-12 will be evaluated for irritation
  - Panel 3: Chips on positions 1-4 and 7-10 will be evaluated for irritation
  - Panel 4: Chips on positions 1-12 will be evaluated for irritation
  - Panel 5: Chips on positions 1-4 and 7-10 will be evaluated for irritation
  - Panel 6: Chips on positions 1-4 and 7-9 will be evaluated for irritation. If Panel 6 is cut to avoid patch testing with the common allergens chips on positions 3-4 and 9 will be evaluated.

Panels 7-8: Irritation attributed to the adhesive used to adhere the panels to the skin (tape irritation) will be documented if the site of the entire panel with the exception of the patch test chambers show signs of irritation. This will be similar to the tape irritation example shown below in Figure 5 but the chambers used in the Finn Chamber product are round, not square.

Figure 5: **has been added.**
Section 10.2, Page 40

The paragraph in:	**has been changed from:**	**To:**
Section 10.3.1, Page 40	Panel 1-6 tape sites (entire panel minus the chip sites) then the polyester chip sites will be evaluated for skin irritation.	Skin irritation due to the patch panel tape or chip will be evaluated and documented per the instructions presented in Section 10.2 Irritation that

		increases in severity or persists beyond visit 3 will be followed to resolution.

The paragraph in:	**has been added.**
Section 10.3.1, Page 42	To ensure that irritation resulting from skin contact with the polyester chip is not confused with an allergic response to a specific allergen, all of the polyester chip sites on each panel must present the same or nearly the same degree of irritation.

The score scale for polyester chip irritation has been added to:
Section 10.4.1, Page 42

The sentence in:	**has been changed from:**	**To:**
Section 10.4.2, Page 42	Categorization of positive reactions as late or persistent; characterized by the presence of hyper/hypo pigmentation and/or pruritus or other described symptoms	All reactions (scores of 1+, 2+ or 3+) will be categorized as either late or persistent. Persistent reactions will be further clarified as either persistent-healing or persistent-escalating. .
The definition in:	**has been changed from:**	**To:**
Section 10.4.2, Page 43	Late Reaction: A reaction that initially appears at day 10-14 after application of panels.	Late Reaction: A reaction that initially appears at day 7-14 after application of panels.
The sentence in:	**has been changed from:**	**To:**
Section 10.5, Page 43	Categorization of positive reactions as late or persistent; characterized by the presence of hyper/hypo pigmentation and/or pruritus or other described symptoms	All reactions (scores of 1+, 2+ or 3+) will be categorized as either late or persistent. Late reactions are defined as reactions that initially occur at 7-10 days after application of the panels. Persistent reactions are defined as reactions that appear at day 2-4 and persist through day 7-21. Persistent reactions will be clarified as either persistent-healing or persistent-escalating.

The following has been added to the illustration of position numbering in:

Figure 7, Page 45	Notations indicating tape notches and an up arrow to indicate product orientation.

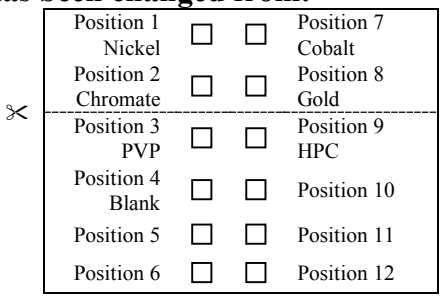
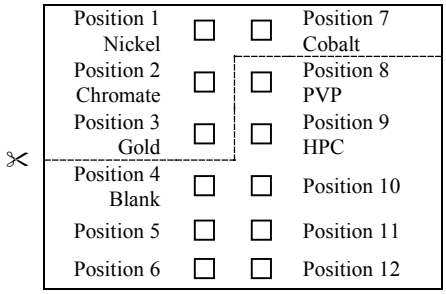
The paragraph following: Figure 7, Page 45	**has been added.** An up arrow and two notches indicate the proper product orientation. The uppermost notch on the left side of the panel indicates the top left of the panel, the lower notch indicates the bottom of the panel. The patch chip numbering is unmistakable when the panel is removed from its pouch. Panels are to be applied with the numbers facing upright.
Figure 8 in: Section 11.1, Page 476	**has been added.** .
The paragraph and table in: Section 11.1, Page 46	**has been added.** In order to differentiate between panels once they are applied the following chip numbers are used for each panel. The *set* of numbers used is unique to each panel so that individual panels can easily be identified: Panel 1: 1-4, 7-10 Panel 2: 13-24 Panel 3: 25-28, 31-34 Panel 4: 25-36 Panel 5: 3-6, 9-12 Panel 6: 1, 4, 5, 7, 8, 12, 28
The paragraph in: Section 11.1, Page 47	**has been added.** The remaining portion of the panel must be applied as it contains the excipient and blank patch controls. Scores for the PVP (Position 3), blank (Position 4) and HPC (Position 9) patches will be recorded in the case report forms as originally intended, in fields for positions 3, 4 and 9. Positions 1, 2 and 7, 8 will be documented in the case report form as 'not applied'.
Figure10 in: Section 11.2, Page 50	**has been added.**
The definitions for hyperpigmentation, hypopigmentaton and pruritus from: Section 10.4.2	**have been moved to:** Section 13.6, Page 56
The sentence from: Section 12.2	**has been removed.** Previous studies have indicated that this number is sufficient for determining an effective allergen dose.
The wording in: Section 13.1, Page 53	**has been added.** or Adverse Drug Reaction (ADR)

The definition in: Section 13.1, Page 53	**has been added.** **Suspected Unexpected Serious Adverse Reactions (SUSAR)** An adverse reaction that is both unexpected (not consistent with the applicable product information) and also meets the definition of a Serious Adverse Event/Reaction.	
The wording in: Section 13.3, Page 54	**has been added.** Serious, unexpected reactions that are not fatal or life-threatening will be filed no later than 15 calendar days after first knowledge by the sponsor.	
The wording in: Section 13.4, Pages 54	**has been changed from:** To comply with worldwide serious adverse event reporting regulations serious, unexpected and related adverse events will be submitted to regulatory authorities according to SmartPractice SOPs.	**To:** Fatal or life-threatening unexpected ADRs will be reported to all regulatory agencies and IRB/Ethics Committees by telephone, facsimile or in writing as soon as possible but no later than 7 calendar days after first knowledge by the sponsor followed by as complete a report as possible within 8 additional calendar days.
The definition in: Section 13.6, Page 56	**has been changed from:** Sensitization: A positive reaction observed 7-14 days after application, with no preceding reaction. The positive reaction must meet the criteria for an allergic reaction (papular or vesicular erythema and infiltration), to help distinguish between a false-positive and sensitization.	**To:** Active Sensitization: A positive reaction initially appearing at approximately 21 days after application. Sensitization may be confirmed if a positive reaction were to appear at 3-4 days after a repeated application of the allergen at a naïve test site
The title in: Section 13.6, Page 56	**has been changed from:** Anaphylactic Reaction	**To:** Acute Anaphylactic Reaction
The sentence in: Section 13.6, Page 56	**has been changed added.** Investigator may refer to the Guideline for Acute Therapy and Management of Anaphylaxis by Ring, et. al for more information	

The definition in: Section 13.6, Page 57	**has been added.** **Tape Reaction:** Severe reaction to the tape or adhesive used to hold the patches in place such that the subject is forced to remove the patch prior to the 48 hour wear time.
The paragraph in: Section 14.0, Page 57	**has been added.** In the event that the study is prematurely terminated or interrupted all regulatory agencies will be notified within 15 days.

21.0 AMENDMENT IV

The Sponsor Assistant Medical Director	**Dathan Hamann, M.D.** has been added to the study.	
The phone for Kayoko Matsunaga, M.D., Ph.D. in: Section 1.0, Page 1	**has been changed from:** 562 93 2339	**To:** 0562 93 9441
Investigator	**Patricia L Norris, M.D.** Oregon Health & Science University	Is no longer participating in the study. She has left her position at Oregon Health & Science University
The IRB for the Dr. Norris site	Oregon Health & Science University IRB	Will no longer be needed due to the departure of Dr. Norris.
The Title for Maki Hosoki, DDS, PhD in: Section 2.0, Page 4 Section 4.0, Page 8	**has been changed from:** Assistant	**To:** Associate
The reference for the Helsinki Declaration in Section 8.2	**has been changed from:** 2000	**To:** 2013
The sentence in: Section 10.1, Page 37	**has been changed from:** Should the Investigator decide that testing with the four common allergens will not be of benefit to the subject, the Investigator will cut Panel 6 below positions 2 and 8 as illustrated in Figure 9, (Section 11.1, Investigational Allergen Panels) before applying	**To:** Should the Investigator decide that testing with the four common allergens will not be of benefit to the subject, the Investigator will cut Panel 6 as illustrated in Figure 9, (Section 11.1, Investigational Allergen Panels) before applying the

	the panel.	panel.
The bullet on Page 38	**has been changed from:** Panel 6: Chips on positions 1-4 and 7-9 will be evaluated for irritation. If Panel 6 is cut to avoid patch testing with the common allergens chips on positions 3-4 and 9 will be evaluated.	**To:** Panel 6: Chips on positions 1-4 and 7-9 will be evaluated for irritation. If Panel 6 is cut to avoid patch testing with the common allergens chips on the remaining positions will be evaluated.
The Sentence on Page 47	**has been changed from:** Should the Investigator elect to omit the common allergens from an individual's patch testing, the panel should be cut below positions 2 and 8 as shown in Figure 9.	**To:** Should the Investigator elect to omit the common allergens from an individual's patch testing, the panel should be cut as shown below in Figure 9.
Figure 9 on Page 47	**has been changed from:** 	**To:** 
The sentence on Page 47	**has been changed from:** Scores for the PVP (Position 3), blank (Position 4) and HPC (Position 9) patches will be recorded in the case report forms as originally intended, in fields for positions 3, 4 and 9. Positions 1, 2 and 7, 8 will be documented in the case report form as 'not applied'.	**To:** Scores for the blank (Position 4), PVP (Position 8), and HPC (Position 9) patches will be recorded in the case report forms as originally intended, in fields for positions 4, 8 and 9. Positions 1, 2, 3 and 7 will be documented in the case report form as 'not applied'.
Panel 6 Common Allergens / Excipient Controls on Page 49	**has been changed from:** PVP Position 3 Gold Position 8	**To:** Gold Position 3 PVP Position 8

22.0 AMENDMENT V

Investigator:	**Prof. Dr. med. Andreas Bircher** FMH in Dermatology/Venereology and Allergology/Clinical Immunology Head of Allergology has retired from the University Hospital Basel Allergology Unit e-mail: andreas.bircher@usb.ch / andreas.bircher@unibas.chand is replaced by: **PD Dr. med. Kathrin Scherer Hofmeier** FMH Dermatology/Venerology and Allergology/Clin. Immunology ad interim Head of Allergology e-mail: kathrin.scherer@usb.ch
The Title for:	**Maki Hosoki, DDS, PhD** has been changed from Associate Professor to Senior Assistant Professor
Investigator	**Akiko Yagami, M.D., Ph.D** Professor, Department of Allergology, Fujita Health University Second Educational Hospital 3-6-10, Otobashi, Nakagawa-ku, Nagoya, 454-8509, Japan has completed all her obligations on the study. The site was officially closed on May 23, 2018.
Sub-Investigator:	Dr. med. Oppel geb. Scharrer, Institute of Dermatology and Allergy has been added to the study.
Clinical Trial Site:	Department of Dermatology Fujita Health University Second Educational Hospital 3-6-10, Otobashi, Nakagawa-ku, Nagoya, 454-8509, Japan will no longer recruit subjects for this clinical trial. The site was officially closed on May 23, 2018.
The IRB:	Institutional Review Board of Fujita Health University Second Educational Hospital Fujita Health University Second Educational Hospital 3-6-10, Otobashi, Nakagawa-ku, Nagoya, 454-8509, Japan Phone: +81-52-323-5774 will no longer be used for this clinical trial. The site was officially closed on May 23, 2018.