



# Standard Test Method for Performing Behind-the-Knee (BTK) Test for Evaluating Skin Irritation Response to Products and Materials That Come Into Repeated or Extended Contact with Skin<sup>1</sup>

This standard is issued under the fixed designation F2808; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 The Behind-the-Knee (BTK) method, using the popliteal fossa of human volunteers as a test site, simultaneously evaluates the inherent chemical irritation, and the potential for mechanical irritation of substrates and products that are designed to come into repeated or extended close contact with the skin (see validation references **1-7**).<sup>2</sup> This is a bilateral test comparing a test material to a reference material with a known safety profile.

1.2 This test method shall be used by qualified health care professionals experienced in good clinical practice (GCP) procedures.

1.3 This test method can be performed using human subjects on either intact or compromised skin. Testing should be performed on intact skin for test substrates or products expected to have contact with normal, intact skin, or for direct comparison to products with a known skin irritation profile. Testing can be performed on compromised skin for test substrates or products that may commonly come into contact with damaged skin (for example, skin with diaper rash, or chapped skin) or skin that is expected to be hydrated.

1.4 Visual scoring of erythema and dryness is performed by a trained skin grader on a pre-defined scale.

1.5 Prior to use in this test, materials shall undergo overall favorable biocompatibility testing consistent with the approach outlined in protocol Practice **F748** or ISO 10993-1:2009. As a part of this series of testing, irritation per Practice **F719** or ISO 10993-10 shall be conducted.

1.6 The values stated in inch-pound units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

## 2. Referenced Documents

### 2.1 ASTM Standards:<sup>3</sup>

**D6355** Test Method for Human Repeat Insult Patch Testing of Medical Gloves

**F719** Practice for Testing Biomaterials in Rabbits for Primary Skin Irritation

**F748** Practice for Selecting Generic Biological Test Methods for Materials and Devices

### 2.2 ISO Standards:<sup>4</sup>

**ISO 10993-1:2009** Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing Within a Risk Management Process

**ISO 10993-10** Biological Evaluation of Medical Devices—Part 10: Tests for Irritation and Delayed-type Hypersensitivity

## 3. Terminology

### 3.1 Definitions:

3.1.1 *chemical irritation, n*—irritation caused by a physiological response to the chemical nature of a material. Such physiological responses may include: oxidation or reduction reactions, dehydration, disruption of the keratin ultra-structure or direct injury to cellular macromolecules or organelles.

3.1.2 *compromised skin, n*—skin that is treated with repeated application of surgical tape prior to the first sample application.

3.1.3 *edema, n*—observable swelling from abnormal accumulation of fluid in connective tissue.

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee **F04** on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee **F04.16** on Biocompatibility Test Methods.

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<sup>2</sup> The boldface numbers in parentheses refer to the list of references at the end of this standard.

<sup>3</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

<sup>4</sup> Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, <http://www.ansi.org>.

3.1.4 *erythema*, *n*—redness of the skin.

3.1.5 *mechanical irritation*, *n*—irritation caused by movement and friction of products intended to remain in contact with the skin for extended periods of time.

3.1.6 *popliteal fossa*, *n*—the area at the back of the knee.

3.1.7 *reference material*, *n*—a material similar in form and composition to the test material. The reference material should have a known safety and irritation profile.

3.1.8 *skin grades*, *n*—visual assessments of erythema and dryness according to a defined scale (see 10.3).

3.1.9 *test material*, *n*—any material or product expected to come into contact with skin.

3.1.10 *trained skin grader*, *n*—personnel who have been trained to reliably recognize erythema and dryness reactions by using reference examples, and by performing side-by-side scoring with an experienced skin grader.

#### 4. Summary of Test Method

4.1 Samples are applied to the back of the knee using an elastic knee band or brace. As the subjects go about their everyday activities, normal movements generate friction between the test sample and the skin at the test site, thereby adding the element of mechanical irritation. Thus, the BTK test protocol evaluates a combination of mechanical irritation and the inherent chemical irritation potential of materials and products that come into contact with the skin.

4.2 This is a randomized, controlled, double blind study in which both the subjects and skin grader are unaware of the treatment assignments. Test and reference materials are applied for 6 h per day, for 5 days. Skin reactions are graded for erythema and dryness prior to the first sample application (that is, at baseline), each morning prior to subsequent sample applications, and upon the removal of the sample at the end of each period.

4.3 Test materials are applied, once each test day to normal, intact skin to evaluate potential reactions to products that normally come into contact with intact skin. To evaluate potential reactions to products that normally come into contact with damaged skin, the skin can be compromised by using tape stripping prior to the first sample application.

#### 5. Significance and Use

5.1 This test method is intended to assess a combination of inherent chemical irritation and mechanical irritation for products and materials expected to come into contact with the skin. It is a comparative approach whereby the potential irritation of a test material is compared to that of a reference material similar in form and composition. The reference material should have a known safety and irritation profile.

#### 6. Interferences and Precautions

6.1 Possible protocol deviations that could interfere with or affect the outcome of the study include the fit of the elastic knee band and the activity level of the subjects. As in any clinical study, adherence to the protocol conditions will offset any potential confounding issues.

6.2 The use of lotions, powders, creams, or skin care products in the skin area at the test sites during the course of the study may impact the study results. In addition, shaving the skin behind the knee, exposing the test sites to tanning or the sun, or swimming or hot tub use during study participation should not be permitted, since these practices and activities may produce low levels of skin irritation in some persons.

6.3 Anti-inflammatory medications may interfere with the normal development of the skin irritation reaction and should not be used during the study.

6.4 Normal showering or bathing cannot be allowed during the sample application of 6 h per day, but can be allowed during the 18 h each day when the test samples are not in place.

6.5 Some persons will experience some degree of skin irritation at the test sites. In addition, some persons may experience slight discomfort from the manner in which the samples are applied (that is, an elastic knee band). In most cases, these effects are anticipated to be reversible when sample application concludes.

#### 7. Apparatus

7.1 An artificial light source, with a 100 W incandescent daylight blue bulb, is used to illuminate the application areas for erythema grading and an illuminated 10× magnifying lens is used to grade dryness.

#### 8. Reagents and Materials

8.1 Elastic knee bands or braces are used to hold the samples in place.

8.2 Test materials include substrates and products that are designed to come into repeated or extended close contact with the skin. The appropriate reference sample is a similar material or product that has been demonstrated through biocompatibility testing to be a non-irritant.

8.3 If testing is to be conducted on compromised skin, an appropriate surgical tape is required.

#### 9. Hazards

9.1 No specific hazards have been identified for this test protocol. However, if any of the test materials tested should fall under biohazard then all used test materials should be handled and discarded by the test site staff following Biohazard Standard Operating Procedures (SOPs).

#### 10. Calibration and Standardization

10.1 No calibration of specific equipment is required.

10.2 Skin graders shall be trained to recognize the degrees of severity of the irritation reactions described in the grading scales given in [Appendix X7](#).

10.3 The Skin Irritation Grading Scales for Erythema and Dryness ([Appendix X7](#)) are similar to those scales used by contract testing facilities in a variety of skin testing protocols, including single patch tests placed on the upper arm, multiple patch tests in cumulative irritation testing, forearm controlled application testing, nasal irritation, and Human Repeat Insult Patch Testing (as outlined in Test Method [D6355](#)). Skin graders

should be trained to recognize erythema and dryness reactions by performing side-by-side scoring of patch tests or BTK tests with an experienced skin grader until the trainee can reliably recognize the reactions.

## 11. Institutional Review and Informed Consent

### 11.1 Institutional Review:

11.1.1 The method for this study and qualification of persons conducting the study shall be reviewed by an appropriate Institutional Review Board (IRB).

### 11.2 Informed Consent:

11.2.1 An informed consent document should be read, understood, and signed by the subjects prior to initiating the study.

## 12. Procedure

12.1 *Subjects*—Each experiment should include a general sample size calculation done by a statistician to determine the number of subjects in the study (see 13.3). A sufficient number of subjects should be recruited give the study sufficient power to achieve a statistically significant difference between the reference and test samples, and to allow for a drop out rate consistent with the norms for the test facility. In the course of recruitment, demographic information (age, sex, ethnicity, height, and weight) should be collected on each subject. Subjects should be healthy adults between 18 and 65 years of age, who meet the inclusion/exclusion criteria and preferably with Fitzpatrick skin type I to IV mixture.

12.1.1 Specific inclusion/exclusion criteria are as follows (see Appendix X1):

### 12.1.1.1 Inclusion Criteria—Subjects who:

- (1) Have read, understood, and signed the informed consent;
- (2) Are at least 18 years old;
- (3) Are no more than 65 years old;
- (4) Are in general good health (self-declared);
- (5) Are Fitzpatrick skin type I to IV (see Appendix X2);
- (6) Agree to refrain from using lotions, creams, powders, or other skin preparations on the skin in the area behind the knee during participation in the study;
- (7) Agree to refrain from shaving the skin in the area behind the knee during participation in the study;
- (8) Agree to refrain from swimming or hot tub use during participation in the study;
- (9) Agree to refrain from fitness training during sample application;
- (10) Agree to refrain from tanning or sun exposure to the test area during participation in the study;
- (11) Agree to refrain from taking anti-inflammatory medication such as acetylsalicylic acid (aspirin), acetaminophen, ibuprofen, and naproxen;
- (12) Agree to comply with all study protocol requirements.

### 12.1.1.2 Exclusion Criteria:

- (1) Fitzpatrick skin type V to VI (see Appendix X2) due to difficulties in scoring visible erythema on highly pigmented skin;
- (2) Cuts, scratches, rashes, sunburn, acne, abrasions, scar tissue, tattoos, or any other skin abnormality at the test sites;

(3) Psoriasis, eczema, skin cancer, or any active dermatitis that could potentially interfere with interpretation of test results at the test sites;

(4) Use of corticosteroids (oral or topical) or other types of drugs that may interfere with the normal development of irritation, inflammation, or immune reactions;

(5) Participation in a behind-the-knee study within the last four weeks;

(6) Known irritancy or discomfort in the area behind the knee which would prevent the subject from wearing a knee brace for 6 h each day during study participation;

(7) Current pregnancy or lactation (self-reported);

(8) Diabetes or kidney disease (self-reported) which may be associated with potential circulatory problems;

(9) Heart or circulatory disease (including blood clots) (self-reported) which may be exacerbated by wearing an elastic knee band for 6 h each day during study participation;

(10) Leg varicosities which would interfere with the subject's wearing a knee brace for 6 h each day during study participation;

(11) Arthritis in the lower extremities;

(12) Known allergies to tape(s) and/or adhesives;

(13) History of edema in the lower extremities;

(14) Knee circumference greater than 21 in. (since 21 in. is the maximum size that can be accommodated by many commercial elastic knee bands).

12.1.2 Once eligibility is confirmed, the areas behind the knees are screened for cuts, scratches, rashes, sunburn, acne, abrasions, scar tissue, tattoos, or any other condition that could prevent a clear assessment of their skin during the test portion of the study.

12.1.3 At the enrollment visit, subjects are fitted for the elastic knee band based on the manufacturer's recommendation. The subject is provided with two identical, unused knee bands to wear throughout the study. However, if a subject is having a degree of discomfort which he or she finds intolerable, adjustments in the size can be made, as long as the identical adjustment is made to both test sites (that is, right and left knee bands). The knee bands are placed in a separate bag for each subject, and labeled in a manner that will identify the subject. One brand of knee bands should be used for all subjects. A sample worksheet is provided in Appendix X3.

12.1.4 Subjects receive an instruction sheet asking them to refrain from using lotions, powders, creams, or skin care products in the area behind their knees during their participation in the study (see sample in Appendix X4). Additionally, they shall be instructed to refrain from shaving the area behind their knees, fitness training during sample application, tanning or sun exposure to the area behind their knees, swimming or hot tub use, or use of anti-inflammatory medications during their participation in the study. Subjects shall be asked to acknowledge compliance with these instructions at each visit, and the responses shall be noted on the grading sheet (see Appendix X5).

12.2 *Preparation and Test Material Application*—The areas behind the knees for each subject are randomly assigned to either the test or reference materials per a randomization

scheme. The sample assignment for each subject is documented and maintained throughout the test.

12.2.1 Test materials include substrates and products that are designed to come into repeated or extended close contact with the skin.

12.2.2 The test can accommodate a variety of sample sizes up to a maximum of 3 in. by 16 in. The test and reference materials can be used as is. Should it be necessary, materials should be manually cut to the same size with clean surgical scissors.

12.2.3 Test materials are placed in approximately the same location behind each knee and held in place using the provided elastic knee bands (see [Appendix X6](#)).

12.2.4 All subjects and the skin grader are blinded to the identity of the test materials.

### 12.3 Daily Procedures:

12.3.1 The *enrollment visit* of the study occurs 4 to 6 days prior to the start of the study (Day –6 to –4). During the enrollment visit, subjects will be asked to sign an informed consent, and complete an inclusion/exclusion questionnaire (sample included in [Appendix X1](#)) by means of an interview conducted by a representative of the test facility. For those subjects enrolled in the study, an initial examination of the test area shall be made. The test sites shall be measured for the elastic knee band, and instructions to the subject reviewed.

12.3.2 *Day 1*—Subjects return to the test facility for the test portion of the study. Compliance to the Subject’s Study Instructions shall be checked verbally.

12.3.2.1 The area behind each knee is scored visually for erythema and dryness by a trained skin grader based on pre-defined scales (see [Appendix X7](#)). This is considered the baseline grade. If a subject exhibits an erythema grade of 2.0 or higher or a dryness grade of 4 or higher on any behind-the-knee site at baseline, the test materials shall not be applied and the subject is excluded from study participation.

12.3.2.2 *Compromising Skin at the Test Sites*—If compromised skin is required, the areas behind the knees are treated by tape stripping (8). One whole piece of an appropriate surgical tape (2 in. wide), should be securely applied to the entire surface area behind-the-knee which is to be covered by the test material, and removed rapidly by pulling it off from the right side of the knee towards the left at around 180°. This procedure should be repeated with fresh strips of tape applied to the same area until an erythema grade of 1.0 to 1.5 is reached. For each subject, the number of tape strippings needed to produce a grade of 1.0 to 1.5 shall be entered on a worksheet. Tape stripping shall be done only on study day 1, prior to the first sample application. (See [Appendix X3](#).)

12.3.2.3 The test materials are applied horizontally to the assigned test area (based on randomization) and held in place with elastic knee bands.

12.3.2.4 Each subject is provided with a Subject Sample Application Time Sheet (see sample in [Appendix X8](#)). The sample application time shall be recorded on the sheet at the test facility. The subject shall be instructed to record the sample removal time on the sheet promptly upon sample removal, and to record any problems experienced at or near the test sites.

12.3.2.5 Subjects shall be instructed to remove the knee bands and test materials at the completion of the 6 hour exposure time, to note the time on the daily diary sheet, and to return to the test facility 30 to 60 min later for the afternoon skin evaluation (the post-patch grade), bringing the knee bands, test samples, and their daily diary sheet with them. The 30 to 60 minute rest period between sample removal and grading allows the skin at the test site to recover from any compression effects that may have resulted from the elastic knee bands. Prolonged compression of the skin may press blood from underlying capillaries, resulting in a whiter appearance. Such compression effects are reversed during the 30 to 60 minute rest period. A wear time equal to or less than 5 h 30 min, or equal to or greater than 6 h 30 min is considered a protocol deviation and should be noted as such.

12.3.2.6 In addition, subjects shall be instructed to remove the elastic knee band(s) at any time during the study when they experience any unusual pain, discomfort or swelling during exposure to the test materials. The subjects should contact the test facility staff immediately to inform them of their decision and reason for removal.

12.3.2.7 At the test facility, skin evaluations are conducted and sample application time sheets are collected. If any test material is considered biohazard then all used test materials shall be discarded by the test site staff as a biohazard, following Biohazard SOPs. The knee bands for each subject shall be returned to the labeled bags.

12.3.3 *Day 2 through 5*—Each subject’s test sites shall be graded for erythema and dryness prior to application of the test materials. This is considered the “recovery grade” (that is, the remaining erythema and dryness after overnight recovery).

12.3.3.1 After the skin grading, the test materials shall be applied in a manner identical to day 1, and held securely in place with the elastic knee band. A new Subject Sample Time Sheet ([Appendix X8](#)) shall be given to each subject for the day.

12.3.3.2 Subjects shall be instructed to remove the knee bands and test materials at the completion of the 6 hour exposure time, and to return to the test facility 30 to 60 min later for skin evaluation (that is, the “post-patch grade”) with all test materials and assigned elastic knee bands. A wear time equal to or less than 5 h 30 min, or equal to or greater than 6 h 30 min is considered a protocol deviation and should be noted as such.

12.3.3.3 At the test facility, skin evaluations shall be conducted and sample application time sheets collected. All used test materials shall be discarded by the test site staff. The knee bands for each subject shall be returned to the labeled bags for use the next day by the same subject.

12.3.4 *Subsequent Visits, if needed*—Any test site showing a visual erythema response of 2.0 or greater, a dryness grade of 4 or greater at the final regularly scheduled grading shall be asked to report for daily grading until the response has regressed to a grade 1.5 or less for erythema, or a dryness grade of 3 or less. In the unlikely event that an erythema reaction of greater than 2.0 persists for longer than 3 days, or at the request of the subject, the subject shall be referred to a dermatologist for treatment.

12.3.5 *Daily Activity Chart*—See [Table 1](#).

TABLE 1 Daily Activity Chart

Table summarizing scheduled procedures	Enrollment visit Day -6 to -4	Day 1	Day 2 through 5	Subsequent visits, if needed
Informed consent form	✓			
Screening questionnaire	✓			
Knee band sizing	✓			
Subject's receive instruction sheet	✓			
Baseline skin grading		✓		
Compromising skin (if applicable) with second grading		✓		
Pre-application skin grading (that is, "recovery grade")			✓	
Sample application		✓	✓	
Each subject receives a sample application time sheet		✓	✓	
Removal of sample by subject (post 6 h wear).		✓	✓	
Interim sample application (if applicable).		✓	✓	
Post-application skin grading (that is, "post-patch grade")		✓	✓	
Collection of knee bands and used test materials		✓	✓	
Collection of completed sample application time sheets		✓	✓	
Follow-up skin grading				✓

12.4 *Removal of Subjects from the Study*—Participation in this study shall be completely voluntary. After admission to the study, the subject may withdraw at any time he or she deems it necessary for any reason and is asked to report such reason fairly and accurately. The Principal Investigator may elect to discontinue a subject's participation at any time he or she deems it necessary or if it is in the subject's best interest. In addition, any subject missing an application or scoring day shall be dropped from the study. The reason for a subject's discontinued participation shall be documented on the Subject Accountability Report Form, such as the sample shown in [Appendix X9](#).

12.5 *Adverse Events*—An adverse event (or adverse experience) is any untoward medical occurrence, such as intercurrent illness or accident, in a study subject administered a test product or material that does not necessarily have a causal relationship with the study interventions. A serious adverse event or serious adverse reaction is any untoward medical occurrence that (1) results in death, (2) is life-threatening, (3) requires inpatient hospitalization or extended hospitalization, or (4) results in persistent or significant disability/incapacity. Any adverse events experienced by any subjects during the study shall be documented using an Adverse Event Report Form, such as the sample shown in [Appendix X10](#).

12.6 In case of subject discontinuation due to voluntary withdrawal, discontinuation by the Principal Investigator, or an adverse event, no replacement subjects will be recruited due to the short duration of this study.

12.7 *Observations and Measurements:*

12.7.1 Skin evaluations shall be conducted by a trained skin grader who is blinded as to the assignment of treatments for each subject. The same grader shall be used for all examinations unless a substitution is necessary in the event of illness or other circumstances. The test materials shall be removed for 30 to 60 min prior to grading so that the grader will not see what test materials the subject was wearing.

12.7.1.1 The primary measurements shall be the erythema and dryness at the test site evaluated according to the scales provided in [Appendix X7](#). The study light source shall be used to illuminate the application areas for erythema grading and the illuminated magnifying lens used to grade dryness. The same trained individual shall score all of the reactions to the test materials during the course of the study.

12.7.1.2 As mentioned previously, if a subject exhibits a visual erythema grade of 2.0 or higher or a dryness grade of 4 or higher on any behind-the-knee site at any of the morning grading sessions, the test materials shall not be reapplied. However, the site shall be graded to the completion of the test to assess the irritation regression response. Any test site showing a visual erythema response of 2.0 or greater, a dryness grade of 4 or greater, and/or any elevated response at the final regularly scheduled grading shall be followed by a daily grading until the erythema response has regressed to grade 1.5 or less, or the dryness has regressed to 3 or less. In the unlikely event that an erythema reaction of greater than 2.0 persists for longer than 3 days, or at the request of the subject, the subject shall be referred to a dermatologist for treatment.

13. **Interpretation of Results**

13.1 At the completion of the study, the mean erythema and dryness for the test versus the reference at each afternoon (that is, post-patch) scoring time point shall be evaluated.

13.1.1 It shall be concluded that the test sample is more irritating than the reference sample if the test sample produces a significantly higher overall mean erythema or dryness.

13.1.2 It shall be concluded that the test and reference samples do not differ in irritation potential if the overall mean erythema and dryness produced by the test and reference samples do not differ significantly.

13.2 For investigative studies, the recovery (that is, morning) scores can provide some additional information on the overall irritation of a material.

### 13.3 Statistical Evaluations:

13.3.1 A statistical model for evaluating erythema and dryness is included in [Appendix X11](#).

13.3.2 Perform a general sample size calculation strategy based on the statistical power for the primary analysis, after the investigator has defined the primary endpoint(s) precisely, formulated the statistical hypotheses, and developed an acceptable modeling and analysis strategy. The power should be calculated for parameter values (means, standard deviations, or incidence rates, in all groups) that are clinically plausible.

13.3.3 If a subject receives an irritation grade  $\geq 2.0$  for erythema or  $\geq 4$  for dryness at any *morning* visit then the subject shall not be re-patched; however, grading shall continue. For statistical purposes in such cases, all subsequent irritation scores shall be set to the grade received immediately after removal of the last patch as long as that grade is higher than any subsequent actual grade. Once a higher actual post-patch removal grade is reached, then the corresponding and subsequent scores for statistical analysis shall be set to that higher grade until a higher actual grade is reported.

13.3.3.1 *Discussion*—To avoid unnecessary discomfort to the test subject, samples are not re-applied at any site with a grade of  $\geq 2.0$  for erythema or  $\geq 4$  for dryness. However, dropping these individuals from the study would result in excluding the highest scores that will likely be recorded in the study, resulting in a bias toward less severe irritation. Likewise, using the results for that subject *only* for those days where the sample is applied to the subject will result in a bias toward a lower overall average score. (See scenario 1 in [Table 2](#).) The physiology of any inflammatory reaction, including skin irritation is that, once the irritation has occurred, it persists or worsens in irritation until the offending agent or condition is removed. Therefore, it is appropriate to assume that those sites where the test material is not reapplied due to excessive irritation would remain irritated or get worse if the material were reapplied. Therefore, the most severe grade for the test

site is carried through to the completion of the study. (See scenario 2 in [Table 2](#).)

## 14. Report

14.1 The report shall contain a description of the test material, demographic data, an accounting of all subjects, the total number of subjects who complete the test, and a description of any protocol variations.

14.2 The report shall contain all individual scores for erythema for each subject, including: baseline (Day 1, morning) and testing phase (Days 1 to 5 afternoon). In addition, the report should contain the mean, the standard error of the mean (SEM) and the standard deviation for the test and reference materials at each scoring timepoint. (Examples are provided in [Appendix X12](#), along with interpretation of the sample results.)

14.3 The report shall contain a summary of any and all statistical analyses conducted on the erythema and dryness scores, with a detailed copy of the analyses included in an attachment.

14.4 The report shall contain a summary of the sample application times (mean  $\pm$  SEM and the range), and a summary of any adverse experiences recorded by the subjects throughout the course of the study. The individual Subject Sample Time Sheets shall be included as an attachment to document these items.

14.5 The report shall contain information on the sample application times for each subject.

## 15. Precision and Bias

15.1 Precision and bias have not yet been determined for this test method.

## 16. Keywords

16.1 clinical study; human testing; mechanical and chemical skin irritation; skin friction; skin irritation

**TABLE 2 Example to Clarify Approach to Inputting Data**

Daily post-baseline AM scores	Day 2	Day 3	Day 4	Day 5	Post-baseline Average
Scenario 1 (Inappropriate scoring) Subject X scores	0.5	1.5	2.0	Sample not applied – no score	average over 3 days, or = $(0.5+1.5+2.0)/3 = 1.33$
Scenario 2 (Recommended scoring) Subject X scores	0.5	1.5	2.0	Sample not applied – scored as 2.0	average over 4 days, or = $(0.5+1.5+2.0+2.0)/4 = 1.75$

**APPENDIXES**

(Nonmandatory Information)

**X1. SAMPLE: INCLUSION / EXCLUSION QUESTIONNAIRE**

X1.1 See Fig. X1.1.

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Check one			INCLUSION CRITERIA
YES	NO		
			1. Has signed a the informed consent?
			2. Is between 18 and 65 years old? Date of Birth: ____ / ____ / ____ mm      dd      yyyy
			3. Is in general good health (self-declared)?
			4. Has Fitzpatrick skin type I – IV (Appendix II)?
			5. Is willing to refrain from using lotions, creams, powders or other skin preparations on the skin area behind the knee during participation in the study?
			6. Is willing to refrain from shaving the skin in the area behind the knee during participation in the study?
			7. Is willing to refrain from swimming/hot tub use during participation in the study?
			8. Is willing to refrain from fitness training during sample applications
			9. Is willing to refrain from tanning/sun exposure to the test area during participation in the study?
			10. Is willing to refrain from taking anti-inflammatory medications, such as: acetylsalicylic acid, acetaminophen, naproxen or ibuprofen, during participation in the study?
			11. Is willing to comply with all study protocol requirements?
Check one			EXCLUSION CRITERIA
YES	NO	N/A	
			1. Has sunburn, acne, abrasions, scar tissue, tattoos or any other skin abnormality at the proposed test sites?
			2. Has psoriasis, eczema, skin cancer or any active dermatitis at the test sites that could interfere with interpretation of test results?
			3. Using corticosteroids (oral or topical)?
			4. Has participated in a behind-the-knee study within the last four weeks?
			5. Has a known irritancy or discomfort in the area behind the knee which would prevent the subject from wearing a knee brace for 6 hours each day during study participation?
female	female	male	6. Is currently pregnant (self-reported)?
female	female	male	7. Is currently lactating?
			8. Has diabetes?
			9. Has kidney disease?
			10. Has heart or circulatory disease (including blood clots)?
			11. Has leg varicosities which would interfere with the subject's wearing a knee brace for 6 hours each day during study participation?
			12. Has arthritis in the lower extremities?
			13. Has known allergies to tapes or adhesives?
			14. Has a history of edema in the lower extremities?
			15. Knee measurement greater than 21 inches.

Based upon dermatologic evaluation and the information contained in this questionnaire, is this subject eligible for inclusion into this study ? Yes      No

--	--	--

If "no", give reason.

---

<b>Principal Investigator's or Designee's Signature</b>	<b>mm</b>	<b>dd</b>	<b>yyyy</b>

**FIG. X1.1 SAMPLE: Inclusion/Exclusion Questionnaire**

**X2. FITZPATRICK'S SKIN TYPE SCALE**

 X2.1 See [Table X2.1](#).

**TABLE X2.1 Sun-Reactive Skin Types  
Fitzpatrick's Classification<sup>A</sup>**

Skin Type	Sensitivity to UV	Sunburn and Tanning History
I	Very sensitive + + + +	Always burns easily; never tans
II	Very sensitive + + + +	Always burns easily; tans minimally
III	Sensitive + + +	Burns moderately; tans gradually and uniformly (light brown)
IV	Moderately sensitive + +	Burns minimally; always tans well (moderate brown)
V	Minimally sensitive + to ±	Rarely burns, tans profusely (dark brown)
VI	Insensitive	Never burns; deeply pigmented (black)

<sup>A</sup> Based on first 30 to 45 min sun exposure after winter season of no sun exposure. Unexposed buttocks skin of individuals of Skin Type I to IV is white, that of Skin Type V is brown, and that of Skin Type VI is dark brown or black.  
From Ref (9).



**X3. SAMPLE: BTK WORKSHEET**

X3.1 See Fig. X3.1.

<b>Subject number</b>					<b>Subject initials</b>		<b>mm    dd    yyyy</b> <b>Date form completed</b>	

LEFT Knee measurement: \_\_\_\_\_ inches  
 RIGHT Knee measurement: \_\_\_\_\_ inches

Elastic knee band: \_\_\_\_\_  
 (Trade name of elastic knee band)

Size of elastic knee band dispensed:

<b>(circle one)</b>	<b>Associated measurement (inches)</b>
Small	
Medium	
Large	

---

**Compromising test sites**

**Does skin need to be compromised for this study?** Yes       No

**If "yes":**      Baseline skin grade for erythema of behind the knee areas to be compromised:

<b>Knee</b>	<b>Erythema Grade</b>	<b>Dryness Grade</b>
<b>L</b>		
<b>R</b>		

Number of tape strips done to LEFT behind-the-knee area: \_\_\_\_\_

Number of tape strips done to RIGHT behind-the-knee area: \_\_\_\_\_

Post-strip skin grade for erythema of behind the knee areas compromised:

<b>Knee</b>	<b>Erythema Grade</b>	<b>Dryness Grade</b>
<b>L</b>		
<b>R</b>		

---

<b>Signature of Skin Lab Coordinator or designee</b>	<b>mm</b>	<b>dd</b>	<b>yyyy</b>

**FIG. X3.1 SAMPLE: BTK Worksheet**

#### **X4. SAMPLE: SUBJECT INSTRUCTIONS**

X4.1 See **Table X4.1**.

**TABLE X4.1 Sample: Subject Instructions**

Thank you for participating in this study. Below is information important to this study.

1. The entire study will last 5 days.
2. The test sites will be graded prior to test material application. Tape stripping may or may not be done to the areas behind the knees prior to test material application on the first day of the study. Test materials will be applied to the area behind your knees and will be held in place with elastic knee bands.
3. You must continue wearing the elastic knee bands and test materials for six hours. Please remove the elastic knee bands and test materials after exactly six hours and record the time of removal.
4. You will need to return to the test site approximately 30-60 min following removal of the test materials and elastic knee bands each afternoon for the skin evaluation. You will return the elastic knee bands and used test products at each afternoon visit. You will return your completed diary questionnaire.
5. Steps 3 and 4 will be repeated on Days 2 through 5.
6. Please refrain from using lotions, powders, creams or skin care products in the skin area behind the knee during your participation in study.
7. Please refrain from shaving the skin area behind the knee during your participation in the study.
8. Please refrain from taking anti-inflammatory medications, such as acetylsalicylic acid, acetaminophen, naproxen or ibuprofen.
9. Please refrain from tanning or sun exposure to the test sites, and swimming or hot tub use during your participation in the study; however, normal bathing practices are permitted.
10. If you experience any swelling in the feet or ankles, remove the knee braces immediately and notify the test facility.
11. If you need to remove the elastic knee bands for any reason prior to the six hours of wear time, or experience any skin reactions other than slight redness or itching, please notify the test facility immediately.

X5. SKIN IRRITATION GRADING SHEET

X5.1 See Fig. X5.1.

**Skin Irritation Grading Sheet**

Subject Number					Subject Initials			

Date	Day of Study	Time of Knee Band Removal	Time of Grading	Knee	Erythema Grade	Dryness Grade	Compliance with instructions?	
							Yes	No
	1: baseline			L				
	1: baseline			R				
	1: afternoon			L				
	1: afternoon			R				
	2: morning			L				
	2: morning			R				
	2: afternoon			L				
	2: afternoon			R				
	3: morning			L				
	3: morning			R				
	3: afternoon			L				
	3: afternoon			R				
	4: morning			L				
	4: morning			R				
	4: afternoon			L				
	4: afternoon			R				
	5: morning			L				
	5: morning			R				
	5: afternoon			L				
	5: afternoon			R				
Date	Extended visit, if needed (i.e., erythema score $\geq 2.0$ or dryness score $\geq 4.0$ on day 5, PM)		Time of Grading	Knee	Erythema Grade	Dryness Grade	Reaction resolved?	
							Yes	No
				L				
				R				

**Compliance with instructions:** Subject refrained from using lotions, powders, creams or skin care products, or shaving the skin area behind the knee. Subject refrained from tanning/sun exposure and swimming/hot tub use. Subject refrained from use of corticosteroids (oral or topical) or other types of drugs that may interfere with the normal development of irritation, inflammatory or immune reactions.

<b>Signature of Grader</b>	mm	dd	yyyy

FIG. X5.1 Skin Irritation Grading Sheet

X6. EXAMPLE OF PLACEMENT OF TEST MATERIALS

X6.1 See Fig. X6.1.



NOTE 1—Test materials are placed horizontally at the behind-the-knee test site, and held in place by an elastic knee band of the appropriate size.

**FIG. X6.1 Test Sample Application in the BTK Test (Ref 5)**

X7. SKIN IRRITATION GRADING SCALES

X7.1 *Skin Irritation Grading Scale—Erythema*—See **Table X7.1**.

X7.2 *Skin Irritation Grading Scale—Dryness*—See **Table X7.2**.

X7.3 *Erythema grading scale described in the below references:*

X7.3.1 Maibach, H., Patrick, E., *Dermatotoxicology*. In: *Principles and Methods of Toxicology*, 4th ed. Edited by A. Wallace Hayes. 2001. CRC Press. p 1067.

X7.3.2 Health Effects Test Guidelines OPPTS 870.2500. Acute Dermal Irritation [http://www.epa.gov/opptsfrs/publications/Test\\_Guidelines/series870.htm](http://www.epa.gov/opptsfrs/publications/Test_Guidelines/series870.htm)

X7.3.3 404 OECD GUIDELINE FOR THE TESTING OF CHEMICALS. Acute Dermal Irritation/Corrosion <http://titania.sourceoecd.org/vl=9806916/cl=25/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s4/p1.idx>

X7.3.4 Ecobichon. Acute Toxicity Studies, In: *The Basis of Toxicity Testing*, CRC Press 1992. p. 52.

X7.3.5 National Research Council. *Principles and Procedures for Evaluating the Toxicity of Household Substances*. National Academy of Sciences, 1977. p. 33.

X7.3.6 Tupker, R. A., Willis, C., Berardesca, E., Lee, C. H., Fartasch, M., Agner, T., and Serup, J., Guidelines on sodium lauryl sulfate (SLS) exposure tests. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis*. 1997 Aug;37(2):53-69.

X7.3.7 ASTM protocol: D 6355-07 Standard Test Method for Human Repeat Insult Patch Testing of Medical Gloves.

X7.3.8 ASTM protocol: F 719-81 Standard Practice for Testing Biomaterials in Rabbits for Primary Skin Irritation.

X7.3.9 Farage, M. A., Gilpin D., Enane, N. A., and Baldwin, S., 2001. Development of a new test for mechanical irritation: behind the knee as a test site. *Skin Res. Technol.* 7: 193-203.

X7.3.10 Farage, M. A., Stadler, A., 2005. Cumulative irritation patch test of sanitary pads on sensitive skin. *J. Cosmet. Dermatol.* 4: 179-183.

X7.3.11 Farage, M. A., 2005. Assessing the skin irritation potential of facial tissues. *Cutan. Ocul. Toxicol.* 24: 125-135.

X7.4 *Skin Dryness grading scale described in:*

X7.4.1 Farage, M. A., 2000. Development of a modified forearm controlled application test method for evaluating the skin mildness of disposable wipe products. *J. Cosmet. Sci.* 51: 153-167.

X7.4.2 Farage, M. A., 2005. Assessing the skin irritation potential of facial tissues. *Cutan. Ocul. Toxicol.* 24: 125-135.

X7.5 *Examples: Skin Irritation Grading Scale Photographs—Erythema*—See **Fig. X7.1**.

X7.6 *Examples: Skin Irritation Grading Scale Photographs—Dryness*—See **Fig. X7.2**.

**TABLE X7.1 Skin Irritation Grading Scale—Erythema**

NOTE 1—The degree of reaction expressed by such descriptive terms as “slight,” “moderate,” and “severe” is, in itself, subjective. Such terminology can be accurately understood only through training and experience.

NOTE 2—Any reaction ≥ Grade 2 for erythema should be described in detail. Unusual reactions not described by the scales below should also be described.

0	No apparent cutaneous involvement.
0.5	Greater than 0, less than 1. <i>Typical example:</i> Faint, barely perceptible erythema or slight dryness (glazed appearance).
1	Faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring.
1.5	Greater than 1, less than 2. <i>Typical example:</i> Well-defined erythema or faint erythema with definite dryness, may have epidermal fissuring.
2	Moderate erythema, may have a few papules or deep fissures, moderate-to-severe erythema in the cracks. <b>If this grade or higher is scored to a test site, the test site will receive no further application of test materials and occlusion.</b>
2.5	Greater than 2, less than 3. <i>Typical example:</i> Moderate erythema with barely perceptible edema or severe erythema not involving a significant portion of the patch (halo effect around the edges), may have a few papules or moderate-to-severe erythema.
3	Severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight edema (edges well defined by raising).
3.5	Greater than 3, less than 4. <i>Typical example:</i> Moderate-to-severe erythema with moderate edema (confined to patch area) or moderate-to-severe erythema with isolated eschar formations or vesicles.
4	Generalized vesicles or eschar formations or moderate-to-severe erythema and/or edema extending beyond the area of the patch.

TABLE X7.2 Skin Irritation Grading Scale–Dryness

NOTE 1—The degree of reaction expressed by such descriptive terms as “slight,” “moderate,” and “severe” is, in itself, subjective. Such terminology can be accurately understood only through training and experience.

NOTE 2—Any reaction  $\geq$  Grade 4 for dryness should be described in detail. Unusual reactions not described by the scales below should also be described.

0	None.
1	Patches of cracking and/or slight powderiness; occasional patches of small scales may be seen, distribution generalized.
2	Generalized, slight powderiness; early cracking or occasional small lifting scales may be present.
3	Generalized, moderate powderiness and/or moderate cracking and scales.
4	Generalized, heavy powderiness and/or heavy cracking and lifting scales. <b>If this grade or higher is scored to a test site, the test site will receive no further application of test materials and occlusion.</b>
5	Generalized, high cracking and lifting scales; eczematous change may be present but not prominent; may see bleeding cracks.
6	Generalized, severe cracking; bleeding cracks and eczematous changes may be present; large scales may be sloughing off.





0	No apparent cutaneous involvement.	
0.5	Greater than 0, less than 1. Typical example: Faint, barely perceptible erythema or slight dryness (glazed appearance).	
1	Faint but definite erythema, no eruptions or broken skin or no erythema but definite dryness; may have epidermal fissuring.	
1.5	Greater than 1, less than 2. Typical example: Well-defined erythema or faint erythema with definite dryness, may have epidermal fissuring.	

FIG. X7.1 Examples—Skin Irritation Grading Scale—Erythema

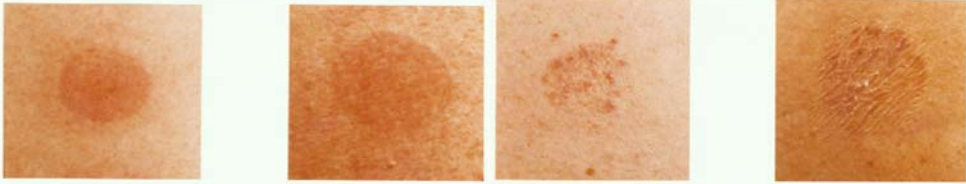




2	Moderate erythema, may have a few papules or deep fissures, moderate-to-severe erythema in the cracks. <b>If this grade or higher is scored to a test site, the test site will receive no further application of test materials and occlusion.</b>
	
2.5	Greater than 2, less than 3. Typical example: Moderate erythema with barely perceptible edema or severe erythema not involving a significant portion of the patch (halo effect around the edges), may have a few papules or moderate-to-severe erythema.
	
3	Severe erythema (beet redness), may have generalized papules or moderate-to-severe erythema with slight edema (edges well defined by raising).
	
3.5	Greater than 3, less than 4. Typical example: Moderate-to-severe erythema with moderate edema (confined to patch area) or moderate-to-severe erythema with isolated eschar formations or vesicles.
	
4	Generalized vesicles or eschar formations or moderate-to-severe erythema and/or edema extending beyond the area of the patch.
	

FIG. X7.1 Examples—Skin Irritation Grading Scale—Erythema (continued)



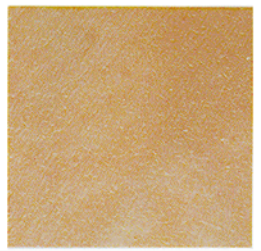


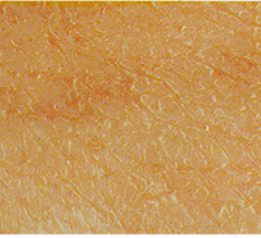

0	None		
1	Patches of cracking and/or slight powderiness; occasional patches of small scales may be seen, distribution generalized.	2	Generalized, slight powderiness; early cracking or occasional small lifting scales may be present.
			
3	Generalized, moderate powderiness and/or moderate cracking and scales.	4	Generalized, heavy powderiness and/or heavy cracking and lifting scales. <b>If this grade or higher is scored to a test site, the test site will receive no further application of test materials and occlusion.</b>
			
5	Generalized, high cracking and lifting scales; eczematous change may be present but not prominent; may see bleeding cracks.	6	Generalized, severe cracking; bleeding cracks and eczematous changes may be present; large scales may be sloughing off.
			

FIG. X7.2 Examples—Skin Irritation Grading Scale—Dryness



**X8. SAMPLE: SUBJECT SAMPLE APPLICATION TIME SHEET**

X8.1 See Fig. X8.1.

<table border="1" style="width: 100%; height: 20px;"> <tr><td style="width: 12.5%;"></td><td style="width: 12.5%;"></td><td style="width: 12.5%;"></td><td style="width: 12.5%;"></td><td style="width: 12.5%;"></td></tr> </table> <p align="center">Subject number</p>						<table border="1" style="width: 100%; height: 20px;"> <tr><td style="width: 33.3%;"></td><td style="width: 33.3%;"></td><td style="width: 33.3%;"></td></tr> </table> <p align="center">Subject initials</p>				<table border="1" style="width: 100%; height: 20px;"> <tr> <td style="width: 33.3%;"></td> <td style="width: 33.3%;"></td> <td style="width: 33.3%;"></td> </tr> </table> <p align="center">mm      dd      yyyy Date form completed</p>			

Date	Day of Study	Time of Knee Band Application	Knee	Time of Knee Band Removal	Did you experience any skin problems at any time in either of the areas being evaluated?	
					Yes	No
	1		L			
			R			
	2		L			
			R			
	3		L			
			R			
	4		L			
			R			
	5		L			
			R			

If yes, please describe the nature of the problem.

Day of Study	
1	
2	
3	
4	
5	

**FIG. X8.1 SAMPLE: Subject Sample Application Time Sheet**

X9. SAMPLE: SUBJECT ACCOUNTABILITY REPORT

X9.1 See Fig. X9.1.

<table border="1" style="width: 100%; height: 20px;"> <tr> <td style="width: 15%;"></td> <td style="width: 15%;"></td> <td style="width: 15%;"></td> <td style="width: 15%;"></td> <td style="width: 15%;"></td> <td style="width: 15%;"></td> </tr> </table> <p style="text-align: center;">Subject number</p>							<table border="1" style="width: 100%; height: 20px;"> <tr> <td style="width: 33%;"></td> <td style="width: 33%;"></td> <td style="width: 33%;"></td> </tr> </table> <p style="text-align: center;">Subject initials</p>				<table border="1" style="width: 100%; height: 20px;"> <tr> <td style="width: 33%;"></td> <td style="width: 33%;"></td> <td style="width: 33%;"></td> </tr> </table> <p style="text-align: center;">mm      dd      yyyy Date form completed</p>			

What is the subject status? (Select One)

Completed Study

Dropped Out

Removed by Investigator

Enter Date Subject Completed Study – or –  
Date Subject Dropped Out of Study:

--	--	--

mm      dd      yyyy

Reason for non-completion (Select One Box Only)

Adverse Event (complete the appropriate Adverse Event Form)

+ Voluntary Withdrawal without an Adverse Event

+ Protocol Violation

+ Change in health status

Study Terminated by Sponsor

+ Other

Died (Complete the detailed Adverse Event Form)

+ Please provide any additional details on reason subject did not complete study:

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Study investigator's or Designee's Signature      mm      dd      yyyy  
Date signed

FIG. X9.1 SAMPLE: Subject Accountability Report

**X10. SAMPLE: SUMMARY ADVERSE EVENT REPORT FORM**

X10.1 See Fig. X10.1.

<input style="width:100%; height: 15px;" type="text"/> <b>Subject number</b>	<input style="width:100%; height: 15px;" type="text"/> <b>Subject initials</b>	<input style="width:100%; height: 15px;" type="text"/> <b>mm dd yyyy</b> <b>Date form completed</b>
---	---	---

**Adverse Event** (diagnosis if available, otherwise symptom): \_\_\_\_\_

---

**Start Date of Event**   
mm dd yyyy

**OUTCOME (select one)**

<input type="checkbox"/> Ongoing	⇒ <b>End date</b> (mm/dd/yyyy)	<input style="width:100%; height: 15px;" type="text"/>
<input type="checkbox"/> Recovered	⇒ <b>Date</b> (mm/dd/yyyy)	<input style="width:100%; height: 15px;" type="text"/>
<input type="checkbox"/> Subject Died		
<input type="checkbox"/> Unknown		

<p><b>SEVERITY (select one)</b></p> <input type="checkbox"/> Mild <input type="checkbox"/> Moderate <input type="checkbox"/> Severe	<p><b>INVESTIGATORS OPINION OF TEST PRODUCT RELATIONSHIP (select one)</b></p> <input type="checkbox"/> Definitely not <input type="checkbox"/> Doubtful <input type="checkbox"/> Possible <input type="checkbox"/> Probable <input type="checkbox"/> Not applicable	<p><b>ACTION TAKEN REGARDING TEST PRODUCT (select one)</b></p> <input type="checkbox"/> None <input type="checkbox"/> Reduced <input type="checkbox"/> Interrupted <input type="checkbox"/> Discontinued <input type="checkbox"/> Not applicable
---	---	--

Was treatment initiated?  Yes  No If 'YES', what? \_\_\_\_\_

Will follow-up be initiated?  Yes  No If 'YES', what? \_\_\_\_\_

**Is this a serious Adverse Event that is immediately reportable to the Sponsor?**  Yes\*  No

\* If yes, select all applicable Seriousness Criteria below and notify the sponsor and IRB within 24 hours.

For all seriousness criteria, except "Withdrawal due to an Adverse Event" (checked exclusively), complete a detailed Adverse Event Report. (**select all seriousness criteria that apply**)

<input type="checkbox"/> Hospitalized or Hospitalization Extended	<input type="checkbox"/> Congenital Anomaly/Birth Defect
<input type="checkbox"/> Life Threatening	<input type="checkbox"/> Medically Significant
<input type="checkbox"/> Persistent/Significant disability/Incapacity	<input type="checkbox"/> Death
	<input type="checkbox"/> Withdrawal due to an Adverse Event

<input style="width:100%; height: 25px;" type="text"/> <b>Study investigator's or Designee's Signature</b>	<input style="width:100%; height: 15px;" type="text"/> <b>mm dd yyyy</b> <b>Date signed</b>
---	---

FIG. X10.1 SAMPLE: Summary Adverse Event Report Form

X11. STATISTICAL MODEL FOR ERYTHEMA AND DRYNESS SCORES

X11.1 The flowchart in Fig. X11.1 describes the process of analysis for by-visit and post-baseline average erythema and dryness grades.

X11.2 If a covariate is significant (p-value  $\leq 0.05$ ), then it can be concluded that the covariate has a significant effect on

irritation and should be included in the reduced model. Assumptions are tested using Shapiro-Wilk for normality and Levene’s test for homogeneity of variance. If either test’s p-value is  $\leq 0.03$ , assumptions are not satisfied.

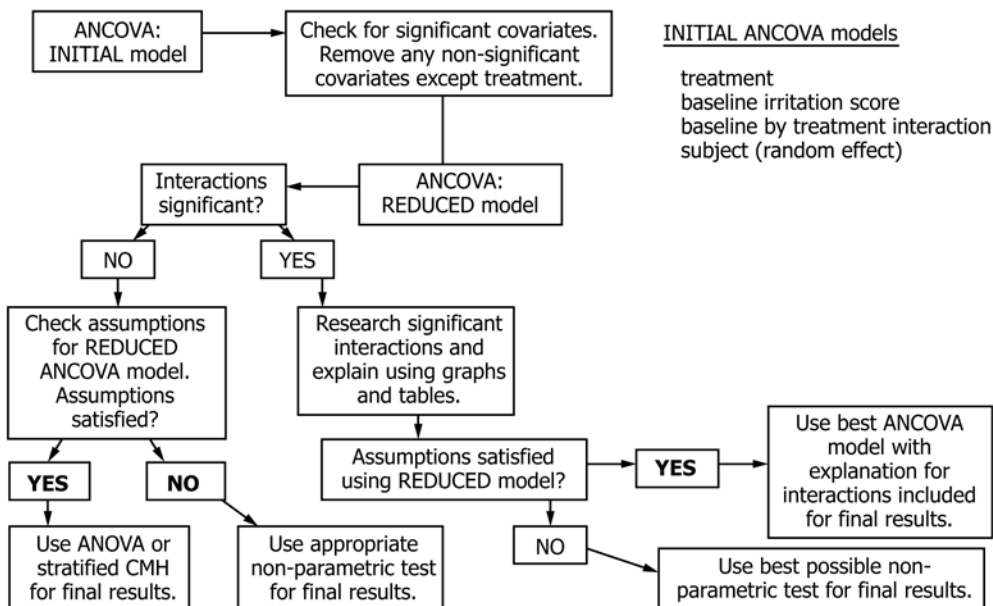


FIG. X11.1 Statistical Model for Erythema and Dryness Scores

**X12. SAMPLE REPORT SUMMARY TABLES**  
(Data for example purposes only)

X12.1 Example 1—See Table X12.1 and Fig. X12.1.

X12.3 Example 3—See Table X12.3 and Fig. X12.3.

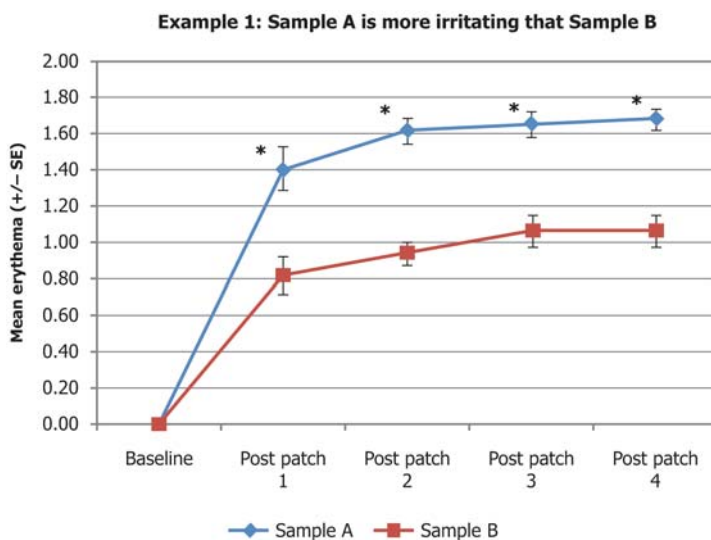
X12.2 Example 2—See Table X12.2 and Fig. X12.2.

**TABLE X12.1 Example 1**

Example 1	Sample A			N	Sample B		Treatment Differences	
	N <sup>A</sup>	Mean ± S.E.	S.D.		Mean ± S.E.	S.D.	Significant	Method
Baseline		0.00 ± 0.00	0.00		0.00 ± 0.00	0.00		
Post patch 1		1.41 ± 0.12	0.48		0.82 ± 0.10	0.39	<sup>B</sup>	CMH test
Post patch 2		1.62 ± 0.07	0.28		0.94 ± 0.07	0.30	<sup>B</sup>	CMH test
Post patch 3		1.65 ± 0.07	0.29		1.06 ± 0.09	0.39	<sup>B</sup>	CMH test
Post patch 4		1.68 ± 0.06	0.25		1.06 ± 0.09	0.39	<sup>B</sup>	CMH test
Overall mean		1.59 ± 0.04	0.34		0.97 ± 0.05	0.38	<sup>B</sup>	ANOVA

<sup>A</sup> The sample size should be calculated based on the statistical power for the primary analysis, after the investigator has defined the primary endpoint(s) precisely, formulated the statistical hypotheses, and developed an acceptable modeling and analysis strategy. In this study, N was equal to 20.

<sup>B</sup> A significantly different from B,  $p \leq 0.05$ .



**FIG. X12.1 Example 1**

**TABLE X12.2 Example 2**

Example 2	Sample C			N	Sample D		Treatment Differences	
	N <sup>A</sup>	Mean ± S.E.	S.D.		Mean ± S.E.	S.D.	p-value	Method
Baseline		0.00 ± 0.00	0.00		0.00 ± 0.00	0.00		
Post patch 1		1.12 ± 0.11	0.45		1.12 ± 0.09	0.38	1.00	CMH test
Post patch 2		1.24 ± 0.09	0.36		1.27 ± 0.11	0.44	0.74	CMH test
Post patch 3		1.44 ± 0.08	0.35		1.44 ± 0.08	0.35	1.00	CMH test
Post patch 4		1.27 ± 0.09	0.36		1.18 ± 0.09	0.35	0.26	CMH test
Overall mean		1.27 ± 0.06	0.23		1.25 ± 0.05	0.22	0.73	ANOVA

<sup>A</sup> The sample size should be calculated based on the statistical power for the primary analysis, after the investigator has defined the primary endpoint(s) precisely, formulated the statistical hypotheses, and developed an acceptable modeling and analysis strategy.

Example 2: Sample C is not more irritating than Sample D

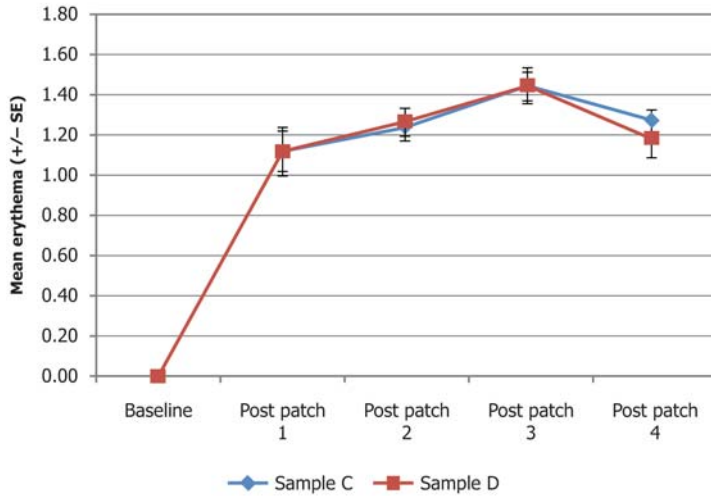


FIG. X12.2 Example 2

TABLE X12.3 Example 3

Example 3	Sample E			N	Sample F		Treatment Differences	
	Mean ± S.E.	S.D.	Mean ± S.E.		S.D.	Significant	Method	
Baseline	0.00 ± 0.00	0.00			0.00 ± 0.00	0.00		
Post patch 1	1.25 ± 0.15	0.68			1.11 ± 0.16	0.75	A	CMH test
Post patch 2	1.43 ± 0.12	0.53			1.15 ± 0.12	0.59		CMH test
Post patch 3	1.25 ± 0.13	0.59			1.24 ± 0.11	0.54		CMH test
Post patch 4	1.34 ± 0.14	0.62			1.33 ± 0.11	0.54		CMH test
Overall mean	1.36 ± 0.10	0.45			1.39 ± 0.09	0.45		ANOVA

<sup>A</sup> E significantly different from F,  $p \leq 0.05$ .

Example 3: Sample E is not more irritating than Sample F

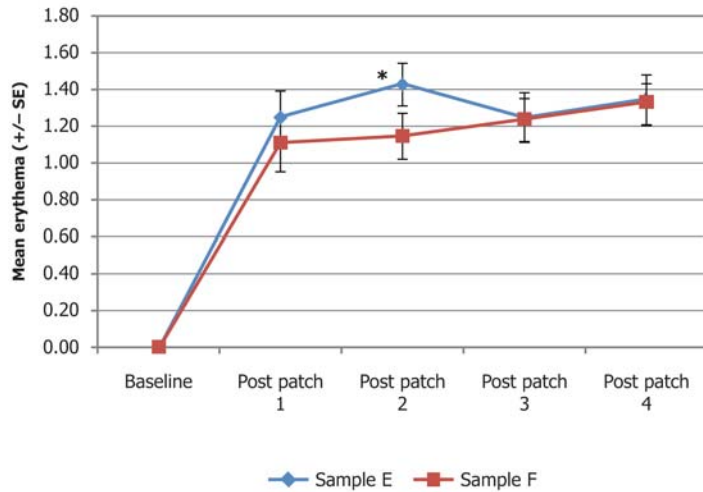


FIG. X12.3 Example 3

## REFERENCES

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