Designation: ~~F2808 – 17~~ F2808 – 23

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[1]

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 (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 The ~~Behind-the-Knee~~behind-the-knee (BTK) method, using the popliteal fossa of human volunteers as a test site, simultaneously evaluates the inherent chemical ~~irritation,~~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**)).[2] 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~~predefined 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~~safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

[1] 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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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

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

F719 Practice for Testing Materials in Rabbits for Primary Skin Irritation

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

2.2 *ISO Standards:*[4]

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~~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.

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~~five 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.

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

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

4.3 Test materials are ~~applied,~~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 or an alternate light source with a similar ~~Coloring Rendering Index~~coloring rendering index (CRI), 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~~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~~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~~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~~institutional review board (IRB) or equivalent ethics review board or committee.

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 to give the study sufficient power to achieve a statistically significant difference between the reference and test samples, and to allow for a dropout 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,~~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~~four to six 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,~~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~~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~~predefined 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 on the affected site. If both behind-the-knee sites are affected then the subject is excluded from study participation. Such grades are recorded and the reason for non-application of the test material(s) ~~is(are)~~ is (are) noted as either "moderate/severe erythema" or "moderate/extreme dryness," respectively.

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),~~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~~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~~ 6 h 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~~ 60 min 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~~min 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~~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~~Day 1, and held securely in place with the elastic knee band. A new ~~Subject Sample Time Sheet~~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~~ 6 h 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~~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~~ 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~~three 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.

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~~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,~~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)~~1) results in death, ~~(2)~~2) is life-threatening, ~~(3)~~3) requires inpatient hospitalization or extended hospitalization, or ~~(4)~~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,~~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,~~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~~three days, or at the request of the subject, the subject shall be referred to a dermatologist for treatment.

TABLE 1 Daily Activity Chart

Table summarizing scheduled procedures	Enrollment visit Day –6 to ~~-4~~ –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				✓

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 ~~≥ 2.0~~ ≥2.0 for erythema or ~~≥ 4~~ ≥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~~reapplied at any site with a grade of ~~≥ 2.0~~ ≥2.0 for erythema or ~~≥ 4~~ ≥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~~Scenario 1 in Table 2.) The physiology of any inflammatory reaction, including skin irritation is that, once the irritation has occurred, it persists or ~~worsen~~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~~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)~~(SEM), and

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

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 ± 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~~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.~~No information is presented about either the precision or bias of Test Method F2808 for screening since the test result is nonquantitative.

16. Keywords

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

APPENDIXES

(Nonmandatory Information)

X1. SAMPLE: INCLUSION / EXCLUSION QUESTIONNAIRE

X1.1 See Fig. X1.1.

Subject number					**Subject initials**			**mm**	**dd**	**yyyy**	
								Date form completed			

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.

Principal Investigator's or Designee's Signature	**mm**	**dd**	**yyyy**

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~~Types—Fitzpatrick's ~~Fitzpatrick's~~ Classification[A]

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)

[A] 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**).