Designation: E1173 – 15

Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations[1]

This standard is issued under the fixed designation E1173; 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 test method is designed to measure the reduction of the microflora of the skin.

1.2 A knowledge of microbiological techniques is required for these procedures.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3.1 *Exception*—In this test method, metric units are used for all applications except for linear measure, in which case inches are used, and metric units follow in parentheses.

1.4 Performance of this procedure requires a knowledge of regulations pertaining to the protection of human subjects (**1**).[2]

1.5 *This standard does not purport to address all of the safety problems, 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:*[3]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E1874 Test Method for Recovery of Microorganisms From Skin using the Cup Scrub Technique

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 Terms used in this standard are defined in E2756, Standard Terminology Relating to Antimicrobial and Antiviral Agents. Others defined below are specific to their use in this document.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *active ingredient, n*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

3.2.2 *inguen, n*—groin: the junctional region between the abdomen and thigh; pl. *inguina*.

3.2.3 *inguinal crease*—the discrete region of flexure between the abdomen and the thigh.

3.2.4 *sampling fluid*—a recovery fluid that contains a neutralizer demonstrated to inactivate or quench the active ingredient(s) in test and reference control formulations. See Test Method E1054.

3.2.5 *test formulation*—a formulation containing an active ingredient(s).

4. Summary of Test Method

4.1 These test methods are conducted on human subjects selected randomly from a group of volunteers who, after refraining voluntarily from using topical and oral antimicrobials for at least two weeks (14 days), exhibit acceptably high normal flora counts on the skin sites to be used in testing (see Section 8).

4.2 The antimicrobial activity of preoperative, vascular precatheterization, or preinjection skin preparations is measured by comparing microbial counts, obtained at various time intervals after application of a test formulation to skin sites, to counts obtained from those same sites prior to application of the test formulation. Skin sites recommended for use in testing are: *(1)* the inguinal region and the abdomen for preoperative skin preparations; *(2)* the inguinal region, the subclavian (clavicular) region, or the median cubital region of the arm for vascular precatheterization preparations, or both; and *(3)* the median cubital region of the arm for preinjection skin preparations.

4.2.1 *Preoperative Skin Preparation*—Microbial samples are collected from the test sites a minimum of three (3) times

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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

[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.

after treatment application on both moist and dry skin sites. The recommended sample times are 10 min, 30 min, and 6 h post-treatment, but other relevant times may be selected.

4.2.2 *Vascular Precatheterization Preparation*—Microbial samples are collected from the test sites a minimum of three (3) times after treatment application on both moist and dry skin sites. The recommended sample times are "immediate," 12 h, and 24 h post-treatment, but other relevant times may be selected. The immediate sample may be 30 s to 10 min, depending on the test material evaluated.

4.2.3 *Preinjection Preparation*—A microbial sample is collected from the test site 30 s post-treatment.

4.3 The fluid used for sampling the test sites must effectively quench (neutralize) the antimicrobial action of all formulations tested. The effectiveness of the inactivator must be demonstrated prior to initiation of product-testing, as described in Test Method E1054, and using in-vivo techniques consistent with the cup-scrub technique (see Section 10).

4.4 To ensure the internal validity of the test, a reference control formulation having performance characteristics known to the laboratory should be tested in parallel with the test formulation.

5. Significance and Use

5.1 These procedures should be used to test topical antimicrobial-containing preparations that are intended to be fast-acting in reducing significantly the number of organisms on intact skin immediately and, for preoperative and vascular precatheterization preparations, to maintain reductions for an extended time.

6. Apparatus

6.1 *Colony Counter*—Any of several types may be used; for example, Quebec colony counters and similar devices, or automated, computerized plater/counter systems.

6.2 *Incubator*—Any incubator that can maintain a temperature of 30° ± 2°C may be used.

6.3 *Sterilizer*—Any steam sterilizer that can produce the conditions of sterilization is acceptable.

6.4 *Timer (stopwatch)*—One that displays hours, minutes, and seconds.

6.5 *Examining Table*—Any elevated surface, such as a 3-by-6-ft (0.9-by-1.8-meter) table with mattress or similar padding to allow the subject to recline.

7. Reagents and Materials

7.1 *Bacteriological Pipettes*—10.0 and 2.2-mL or 1.1-mL capacity, available from most laboratory supply houses.

7.2 *Petri Dishes*—100 mm by 15 mm, for performing standard plate counts, available from most laboratory supply houses.

7.3 *Scrubbing Cups*—Autoclavable cylinders, height approximately 1 in (2.5 cm), inside diameter of a size convenient to placement on the skin of the anatomical area to be sampled. Useful diameters range from approximately 0.5 to 1.5 in (1.3 to 3.8 cm), depending on sites to be sampled.

7.4 *Rubber Policeman, TFE-fluorocarbon Scrubbers, or other appropriate advice*—Can be fashioned in the laboratory or purchased from most laboratory supply houses. Whichever type is selected, it should be used throughout the course of testing.

7.5 *Testing Formulation, including directions for use.*

7.6 *Sterile Gauge Pads*—Used to cover treated skin sites.

7.7 *Sterile Dressings*[4]—Used to cover treated skin sites.

7.8 *Sampling Fluid*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, and 1.0 g isooctylphenoxypolyethoxyethanol in 1 L of distilled water. Inactivator(s) specific for the antimicrobial active(s) in the test and reference control formulations must be included (See Test Method E1054). Adjust to pH 7.8. Dispense in appropriate volumes and sterilize.

7.9 *Dilution Fluid*—Butterfield's **(2)** phosphate-buffered water adjusted to pH 7.2, or other suitable diluent, which must contain antimicrobial inactivators specific for the test and reference control formulations (see Test Method E1054).

7.10 *Plating Medium*—Soybean-casein digest agar **(3)**, with or without antimicrobial inactivators.

7.11 *Sterile Template Material*—Used to demarcate the skin sites; made from paper, plastic, or cloth, for example.

7.12 *Surgical Skin Marker*—Used to delineate mark the skin sites to be used in testing.

NOTE 1—Because some markers contain crystal violet or other fluids that are inhibitory to many skin microflora, a marker should be proven non-antimicrobial prior to use in testing.

8. Skin Sites to be Used in Testing

8.1 *Preoperative Skin Preparations:*

8.1.1 The skin sites selected for evaluation of the effectiveness of preoperative skin preparations should include both moist and dry skin areas. Bacterial baseline populations should be at least 3.0 $\log_{10}/cm^2$ greater on moist skin sites than the detection limit of the sampling procedure, and at least 2.0 $\log_{10}/cm^2$ greater than the detection limit on dry skin sites. The preferred moist-skin areas are the inguina, in which skin-to-skin contact results in a moist environment conducive to higher populations of microflora. The preferred dry-skin area is the lower abdomen below the umbilicus. These areas are illustrated in Fig. 1.

8.1.2 Using a 1.5-by-5-in (3.8-by-12.7-cm) sterile template (for example, paper, plastic, cloth), treatment sites in the inguina are delineated on the uppermost inner aspects of both thighs, centering the long axis of the template along the inguinal crease, and marking the corners using a surgical skin marker. If, due to a subject's anatomy, the treatment site cannot be centered along the inguinal crease, the site should be

[4] The sole source of supply of the apparatus (TELFA non-adherent dressing, No. 3279) known to the committee at this time is Kendall Co.; Hospital Products; Boston, MA 02101. This product is not sterile, but can be steam-sterilized prior to use. If you are aware of alternative suppliers of appropriate dressings, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

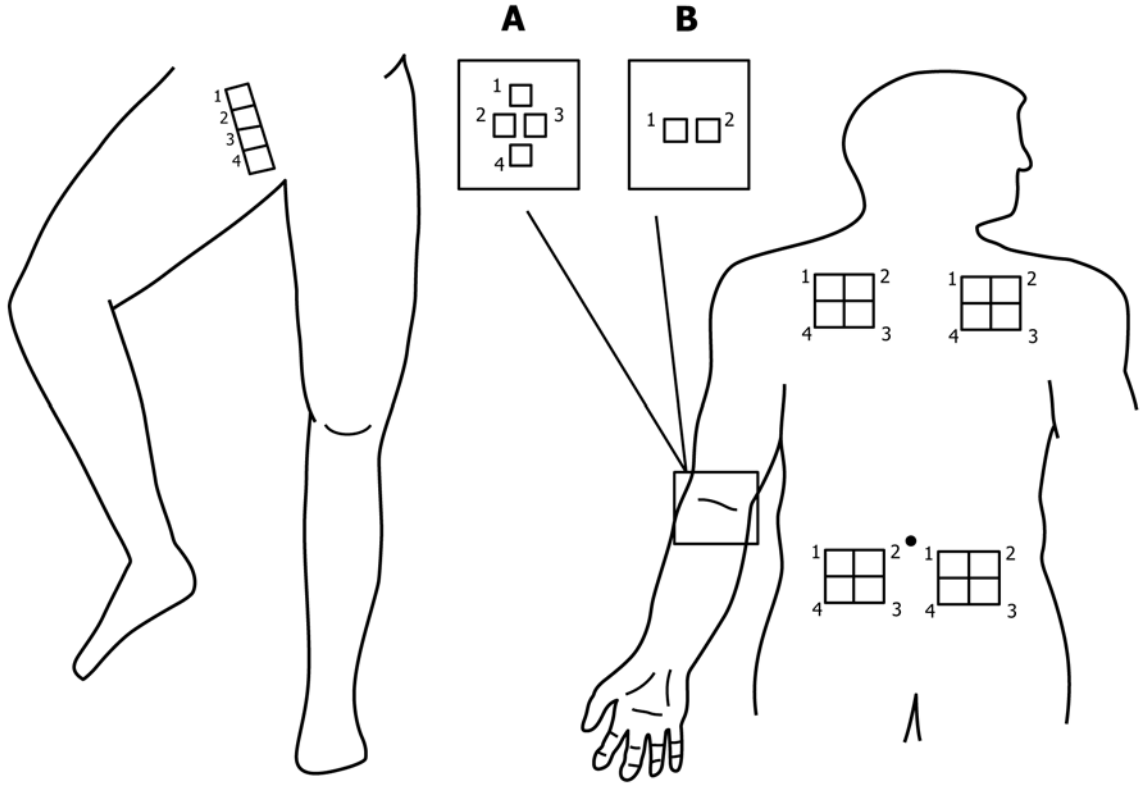

FIG. 1 Illustration of Approximate Sampling Locations on Treatment Sites: Inguen, Abdomen, Clavicular Region, and Median Cubital Region of Arm

positioned on the upper, inner thigh as close to the crease as possible. In no instance should testing be performed on areas not having skin-to-skin contact. The site is then divided on the long axis into 1-by-1.5-in (2.5-by-3.8-cm) sampling areas, allowing for spaces of about 0.25 (about 0.6 cm) between each of the four areas.

8.1.2.1 Sampling areas are numbered from anterior to posterior, beginning with 1 and proceeding perineally to 4, and then are randomized to sampling for baseline and the three post-treatment sampling times (see Note 2).

NOTE 2—Bacterial populations in the inguina are known to be heterogeneous, with counts tending to increase proceeding from the upper reaches of the inguinal crease perineally toward convergence of the inguina at the gluteal fold, and to decrease proceeding laterally from the inguinal crease onto the (dry) surface of the upper thigh. Hence, sampling areas must be confined to skin immediately adjacent to the inguinal crease where skin-to-skin contact provides the moist environment conducive to bacterial growth. Note that the large variance in the count data that results from randomization of the sampling areas likely will require testing of a relatively large number of subjects in order to demonstrate statistical significance of post-treatment reductions.

8.1.2.2 Because of constraints imposed by the anatomical area, sampling cylinders used for the inguinal sites must be ≤ 1 in (≤ 2.54 cm) in diameter.

8.1.2.3 The test formulation and reference control material are then randomized bilaterally to the treatment sites.

8.1.3 Abdominal treatment sites are to be located within 5-by-5-in (12.7-by-12.7-cm) sites below and to the right or left of the umbilicus, approximately midway between the umbilicus and the pubis. Using a 5-by-5-in (12.7-by-12.7-cm) sterile template (for example, paper, plastic, cloth), the corners of each site are numbered 1, 2, 3, and 4 directly on the skin, using a surgical skin marker. Numbering is to be the same for all abdominal sites: number 1 is placed at the top corner to the subject's right, and numbers 2, 3, and 4 are assigned in order clockwise from 1. Three quadrants of each site are used for the three different treatment exposure times, and the remaining quadrant is used for a baseline count. The test formulation and reference control material are then randomized to the treatment sites, right and left, and baseline and the three post-treatment sampling times are randomized to the four sampling areas within each site.

8.2 *Vascular Precatheterization Skin Preparations:*

8.2.1 The skin sites selected for evaluation of the effectiveness of vascular precatheterization skin preparations should include body areas that may be catheterization sites and should include both moist and dry skin areas. Bacterial baseline populations should be at least 3.0 $\log_{10}/cm^2$ greater on moist skin sites than the detection limit of the sampling procedure, and at least 1.0 $\log_{10}/cm^2$ greater than the detection limit on dry skin sites. The preferred moist-skin areas are the inguina, and the preferred dry-skin areas are the clavicular region and the median cubital region of the arm.

8.2.2 Test sites in the inguina are to be located and evaluated as specified for testing of preoperative skin preparations (see 8.1.2.1, Note 2, and Fig. 1).

8.2.3 The dry skin sites and sampling configurations used in testing vascular precatheterization preparations are illustrated

in Fig. 1 and Fig. 1 Detail A. Sterile templates (for example, paper, plastic, cloth) are fashioned for the sampling configuration such that they accommodate the diameter of the sampling cylinder, plus at least 0.5 in (1.25 cm) between the 4 sampling areas. The template is applied to the treatment site, and a surgical skin marker is used to demarcate the sampling areas. These are numbered 1 through four at outside corners, beginning at the subject's upper right and proceeding clockwise in the clavicular region, and beginning proximally and proceeding distally on the arm. Three sampling areas of the site are used for different treatment exposure times of "immediate" (30 s to 10 min, depending on test product), 12 h, or 24 h, and the remaining sampling area is used for a baseline count. The test formulation and reference material should be randomized to the treatment sites, right or left, and exposure times and baseline should be randomized to the four quadrants of each site.

8.3 *Preinjection Skin Preparations:*

8.3.1 The skin site selected for use in evaluating the effectiveness of preinjection skin preparations should represent a body area that is commonly used for transepidermal injection or phlebotomy. Bacterial baseline populations should be at least 1.0 $\log_{10}/cm^2$ greater than the detection limit of the sampling procedure. A suitable dry-skin area is the median cubital region of the arm.

8.3.2 The dry-skin site and sampling configuration used in testing preinjection preparations are illustrated in Fig. 1 Detail B. Sterile templates (for example, paper, plastic, cloth) are fashioned for the sampling configuration, such that they accommodate the diameter of the sampling cylinder, plus at least 0.5 in (1.25 cm) between the two sampling areas. The template is applied to the treatment site, and a surgical marker is used to demarcate the sampling areas. These are numbered 1 and 2 at outside corners. One sampling area of the site is used for the treatment exposure of 30 s, and the remaining sampling area is used for a baseline count

8.3.3 The test formulation and reference control material are then randomized to the treatment sites, right and left, and baseline and post-treatment or baseline are randomized to the two sampling areas.

9. Procedure

9.1 *Number of Subjects*—Because the purpose of the study is to demonstrate efficacy (defined as a significant reduction from baseline counts), sample size calculations should be done to determine the number of subjects per treatment group necessary to find statistically significant differences (reductions) from baseline. The number of subjects required depends on the statistical confidence required for the expected results, the variability encountered in the data collection (for example, variability in reductions from baseline), and the expected efficacy of the test product (for example, approximate reductions from baseline expected). This number of subjects per treatment group (n) can be estimated from the following equation (**4**):

$$n \geq S^2\left[\frac{(Z_{a/2}+Z_{\beta})^2}{D^2}\right]$$

where:

S^2 = estimate of variance (of reductions from baseline based on in-house data pool);
$Z_{a/2}$ = cumulative probability of the standard normal distribution = 1.96 for α = 0.05;
Z_{β} = power of the test = 0.842 for ß = 0.80; and
D = expected efficacy (expected reduction from baseline).

9.2 Recruit a sufficient number of healthy adult volunteers who have no visual evidence of dermatoses, open wounds, or other skin disorders that may affect the test.

9.3 *Pretest Period (14 days)*—Instruct volunteers selected as test subjects to avoid contact with antimicrobials (other than test formulations) for the duration of the pretest and test periods. This restriction includes antiperspirants, deodorants, shampoos, lotions, bathing soaps, body powders, other hygienic products that contain antimicrobials, and such materials as acids, bases, and solvents. Subjects also are to refrain from wearing clothes that have been treated with antimicrobials or fabric softeners, and from bathing in biocide-treated pools, hot tubs, or spas.

9.3.1 Provide test subjects with a kit of nonantimicrobial personal hygiene products for exclusive use during the pretest and test periods. Subjects are not to shower or tub-bathe during the 24-h period prior to the application of test material or microbial sampling. The bathing restriction period may be lengthened, if desired, to increase bacterial populations.

9.3.2 If the skin sites selected for testing include areas that would require clipping of hair prior to surgery (for example, the abdominal and inguinal regions), hair from these sites should be clipped to reduce difficulties bandaging them. Clipping must be performed at least 48 h prior to microbial sampling for first baseline.

9.4 *Test Period*—After subjects have refrained from using antimicrobials for at least two weeks, obtain at least one estimate of baseline bacterial population from the anatomical regions to be evaluated. Collect these samples at least 72 h prior to initiation of product-testing to permit selection of only those subjects whose baseline counts meet the minimum criteria for the treatment site (see 8.1.1, 8.2.1, or 8.3.1). Sampling and enumeration techniques described in Sections 10 and 11 should be applied.

9.4.1 A baseline sample is to be collected at the time of testing. The sample should be taken from a sampling area predetermined by design (for example, on an inguinal site) or by random assignment to sampling areas within a treatment site.

9.4.2 *Treatment Application Procedure*—Immediately after taking the baseline sample, the treatment is applied according to label directions or as stated in the proposed directions.

9.4.3 *Sampling Schedule*—According to the predetermined sampling design or randomization, samples of the prepped site are taken from the sampling areas when the specified post-treatment exposure times have elapsed.

NOTE 3—Between the time of treatment application and final sampling, subjects should avoid activities or positions that would cause untreated skin sites or clothing to contact treated sites. To allow the subjects some degree of mobility between the time of treatment and final sampling, the treated skin areas should be covered with a sterile semi-occlusive dressing

(7.6 and 7.7). This material is applied in a manner so as to protect the treated skin site from contact with untreated skin, and such that air circulation is not restricted.

10. Microbiological Sampling Methods

10.1 Microbial samples are obtained using the cup scrub technique. See (**5**) and Test Method E1874. At the designated sampling time, a sterile cylinder (glass or stainless steel) is held firmly onto the area to be sampled. A known volume of sterile sampling fluid containing appropriate product inactivators is instilled into the cylinder. The known volume selected will depend on the size of the cylinder and the total amount of fluid required for subsequent dilution. The skin area inside the cylinder is then massaged in a circular manner for 1 min with a sterile rubber policeman, Teflon scrubber, or other appropriate device. At the end of the timed procedure, the fluid is removed and placed in a sterile test tube.

10.2 A second known volume of sterile sampling fluid containing appropriate product inactivators is instilled into the cylinder, and the skin area inside the cylinder is massaged again in a circular manner for 1 min with the scrubbing device. At the end of the timed procedure, the fluid is removed and placed in the test tube with the first aliquot, pooling the samples.

10.3 1.0 mL aliquots of this suspension of microorganisms (10^0 dilution) are removed and serially diluted in Butterfield's[3] phosphate-buffered water, or other suitable diluent containing product inactivators. Serial diluting and plating should be completed within 30 min.

11. Enumeration of Bacteria in Sampling Solution

11.1 The bacteria in the sampling fluid are enumerated using standard procedures such as those described in Wehr and Frank (**6**), but using soybean-casein digest agar (7.10) and a suitable inactivator for the antimicrobial, where necessary. Sample dilutions are analyzed in duplicate and are incubated at 30° ± 2°C for 48 to 72 h before enumeration.

11.2 In order to convert this volumetric measurement into the number of colony-forming units per square centimeter (cm^2), the following formula is employed:

$$R = \log_{10}\left[\frac{F \times \left[\frac{\sum c_i}{n}\right] \times 10^{-D}}{A}\right]$$

where:

R = the average colony-forming unit count in $\log_{10}$ scale per cm^2 of sampling surface;
F = total number of mL of stripping fluid added to the sampling cylinder (refer to Section 10);
$\Sigma c_i/n$ = average of the duplicate plate counts used for each sample collected;
D = dilution factor of the plate counts; and
A = inside area of the cylinder in cm^2.

12. Study Design

12.1 The basic study design for these testing procedures is a pre- to post-treatment comparative structure. The microbial population determined prior to treatment with test formulation (baseline population) is compared with the population remaining at a specified time after treatment (**7**).

NOTE 4—Baseline microbial population counts for the left and right test sites should be pooled, but only if they are shown to be statistically equivalent at a ≤ 0.05. This permits direct comparison of formulations on the basis of a single mean baseline value.

12.2 The basic design for a study of two formulations is illustrated as follows (**8**):

Pre-Treatment			Post-Treatment				
R (1)	O_{BL1}	O_{BL2}	A (1)	$O_{1,1}$	$O_{1,2}$	...	$O_{1,n}$
R (2)	O_{BL1}	O_{BL2}	A (2)	$O_{2,1}$	$O_{2,2}$	...	$O_{2,n}$

where:

R = test sites, right and left, assigned randomly to the test and reference formulations: where I = 1, if formulation 1; or 2, if formulation 2.
A = Independent variables: test and reference formulations.
O_{ij} = Dependent variables: microbial counts.
i = BL, if Baseline 1, 2, ...n; 1, if formulation 1; and 2, if formulation 2; and
j = exposure times 1, 2, ...n

12.3 This design will accommodate statistical evaluation by means of either parametric or nonparametric models.

13. Statistical Analysis

13.1 Regardless of the analytical model selected, it is preferable that the level of significance be set at α = 0.05 for rejection of H_o, the hypothesis of no significant difference between microbial populations, pre- to post-treatment.

13.2 Student's *t* test is an appropriate parametric approach to the analysis of the changes from baseline (pre- to post-treatment). It may be appropriate to adjust the alpha level due to multiple *t* tests being conducted (**9**). One choice for an adjusted value of alpha (α*) is calculated as follows:

$$\alpha^* = 1 - (1 - \alpha)^k$$

where:

k = the number of comparisons to be made;
α = the level of significance desired (= 0.05, preferably); and
α* = true level of significance for each of the multiple tests on H_o.

13.3 If data do not conform to a normal distribution, a nonparametric statistical model, such as the Mann-Whitney U Test, may be applied.

14. Precision and Bias

14.1 A precision and bias statement cannot be made for this test method at this time.

15. Keywords

15.1 antimicrobial; cup scrub; efficacy; precatheterization; preinjection; preoperative; skin preparation

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