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# Standard Test Method for Strength Properties of Tissue Adhesives in Tension[1]

This standard is issued under the fixed designation F2258; 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 This test method is intended to provide a means for comparison of the adhesive strengths of tissue adhesives intended for use as surgical adhesives or sealants, or both, on soft tissue. With the appropriate choice of substrate, it may also be used for purposes of quality control in the manufacture of tissue adhesive based medical devices.

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

1.3 *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:*[2]
D907 Terminology of Adhesives
E4 Practices for Force Verification of Testing Machines
2.2 *American Association of Tissue Banks Standards:*[3]
Standards for Tissue Banking

## 3. Terminology

3.1 *Definitions*—Many terms in this test method are defined in Terminology D907.

3.2 *Definitions:*

3.2.1 *tissue adhesive*—for the purposes of this test method, tissue adhesive is defined as a compound or system intended for use in closing wounds (surgical or traumatic) or for sealing against leakage of body fluids.

3.2.2 *tissue sealant*—a surface coating with adequate adhesive strength to prevent leakage of body fluids.

## 4. Significance and Use

4.1 The utility, range, and efficacy of adhesives in clinical medicine are well documented in the literature. Whether being used as an adhesive, hemostatic, sealant, or carrier for drugs or growth factors, or both, the scope of adhesive use in clinical medicine continues to expand. There are several factors which are vital to the success and efficacy of a medical tissue adhesive including, (*1*) adequate tissue bonding strength, (*2*) tissue compatibility, (*3*) acceptable biodegradable properties when the adhesive is used internally, (*4*) availability, (*5*) ease of application, and (*6*) cost.

4.2 Medical adhesives are currently used for a variety of applications and tissue types. Applications range from fixation of external tissues to internal application for use with either similar or dissimilar opposing surfaces. While the biological or chemical makeup, or both, of the adhesive may define its characteristics, additional mechanical factors including adhesive volume or method of application, or both, may also contribute significantly toward the performance of the adhesive. In an effort to fairly and adequately quantify adhesive bonding strength for medical adhesives, it is important to develop a consistent, reproducible testing standard for evaluative and comparative purposes. Due to the fact that the adhesives will be used on or in living tissues, a readily available biological testing surface is preferred.

4.3 The data generated from a standardized testing method on biologic tissue may vary from that found *in vivo*, however, testing results should offer valuable information on the potential bonding capacity and for the preparation of subsequent *in vivo* experiments.

4.4 The complexity and variety of individual applications for tissue adhesive devices, even within a single indicated use (surgical procedure), is such that the results of a tensile test are not suitable for determining allowable design stresses without thorough analysis and understanding of the application and adhesive behaviors.

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[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 F04.15 on Material Test Methods.

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[2] 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.

[3] Available from the American Association of Tissue Banks (AATB), 1350 Beverly Rd., Suite 220-A, McLean, VA 22101.

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4.5 This test method may be used for comparing adhesives or bonding processes for susceptibility to fatigue and environmental changes, but such comparisons must be made with great caution since different adhesives may respond differently to varying conditions.

## 5. Apparatus

5.1 *Testing Machine,* of the constant-rate-of-crosshead-movement type and comprising essentially the following:

5.1.1 *Fixed Member,* a fixed or essentially stationary member carrying one grip.

5.1.2 *Movable Member,* a movable member carrying a second grip.

5.1.3 *Grips,* for holding the test specimen between the fixed member and the movable member of the testing machine can be either the fixed or self-aligning type.

5.1.3.1 *Fixed Grips* are rigidly attached to the fixed and movable members of the testing machine. When this type of grip is used, extreme care should be taken to ensure that the test specimen is inserted and clamped so that the long axis of the test specimen coincides with the direction of pull through the centerline of the grip assembly.

5.1.3.2 *Self-aligning Grips* are attached to the fixed and movable members of the testing machine in such a manner that they will move freely into alignment as soon as any load is applied so that the long axis of the test specimen will coincide with the direction of the applied pull through the center line of the grip assembly. The specimens should be aligned as perfectly as possible with the direction of pull so that no rotary motion that may induce slippage or damage to the sample will occur in the grips; there is a limit to the amount of misalignment self-aligning grips will accommodate.

5.1.4 *Drive Mechanism,* for imparting to the movable member a uniform, controlled velocity with respect to the stationary member, with this velocity to be regulated as specified in 9.3.

5.1.5 *Load Indicator,* a suitable load-indicating mechanism capable of showing the total tensile load carried by the test specimen when held by the grips. This mechanism shall be essentially free of inertia lag at the specified rate of testing and shall indicate the load with an accuracy of ±1 % of the indicated value, or better. The accuracy of the testing machine shall be verified in accordance with Practices E4.

5.2 *Temperature-controlling Equipment,* capable of maintaining the test temperature to ±2°C. If ambient laboratory conditions are employed, the same degree of control is required. A water bath or environmental chamber capable of maintaining 37°C is required for testing on tissue substrates.

## 6. Test Substrate

6.1 For comparative testing, either fresh or frozen split thickness porcine skin graft may be used.

6.1.1 Frozen split thickness porcine skin that has been aseptically prepared is available commercially and is preferred due to ease of use and the potential for more consistent properties. It should be thawed according to the manufacturer's instructions prior to use. Unused graft may be kept at 2 to 8°C for up to two weeks after thawing.

6.1.2 If fresh skin is chosen, it should be prepared according the method in Appendix X1.

6.2 *Application Specific Testing:*

6.2.1 The strength of any adhesive is highly dependent on the test substrate, or adherend. For a specific application, the preferred substrate is freshly harvested tissue from the target organ of a domestic food animal. Tissue from bovine, porcine, or ovine origin is preferred due to wide availability and the fact that relatively large samples of tissue can be harvested from a single source. Ideally, the tissue should be used within 24 h of harvest, and should be kept between 5 and 10°C prior to testing if it cannot be used immediately after harvesting. Storage and handling of tissue samples should be carried out according to the guidelines set forth in Standards for Tissue Banking by the American Association of Tissue Banks. The specimens should be brought to the test temperature or other prescribed temperature (such as body temperature) prior to application of the adhesive.

6.2.2 Fixed tissue should not be used since it has been demonstrated that fixatives cause large alterations in the mechanical properties of the tissue and it is probable that the adhesive strength would be affected as well.

6.2.3 If the target organ is of a size or geometry, or both, that does not allow fabrication of test samples as shown in Fig. 1, a tissue of similar origin but larger size should be used. For example, if the intended indication is for anastomosis of small blood vessels, a larger vessel should be substituted.

6.2.4 The thickness of the tissue sample should be minimized and should not exceed 5 mm. Thicker samples will lead to distortion of the substrate and mixed loading (shear and tension). It is also important that the thickness be as uniform as possible.

6.3 *Substrates for Quality Control Testing:*

6.3.1 For testing that is undertaken as part of a quality control process in the manufacturing of a tissue adhesive device, the use of freshly harvested tissue is highly inconvenient and may also lead to unacceptable variation in the test results, especially if the failure occurs in the adherend (substrate failure). Since the purpose of quality control testing is to

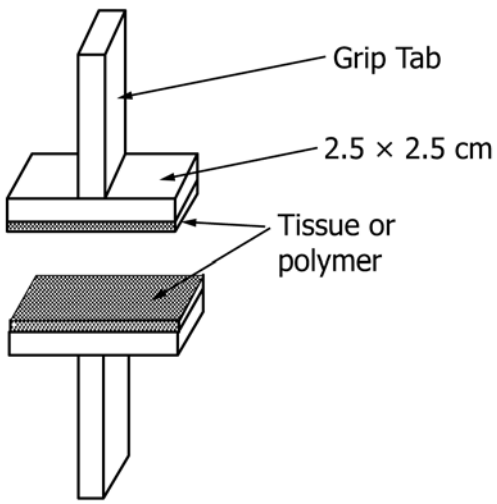


**FIG. 1 Test Fixtures**

demonstrate consistency in the device, substitution of a model substrate is preferred so long as it is demonstrated that the adhesive does bond to the adherand. For devices that require contact with tissues to cure, Mediskin XenoGraft should be used for quality control testing as well as comparative testing.

## 7. Test Specimen

7.1 *Specimens with Soft-tissue Substrates* shall conform to the form shown in Fig. 1. The only critical dimension is the bonding surface, which shall be 2.5 ± 0.005 $cm^2$. The tissue can be bonded to the specimen holder with any suitable adhesive. Gel-type cyanoacrylate adhesives have been found to be convenient for this purpose since they adhere well to moist tissues and cure quickly. In cases where the test adhesive is based on cyanoacrylates, this test method may not work with all tissue types since the tissue may pull off of the fixture instead of failing at the test adhesive interface. In this case, alternative means of securing the tissue to the test fixture may need to be employed or an alternative test configuration such as T-Peel or Lap-Shear can be chosen.

7.2 *Specimens with Polymer or Metal Substrates* shall conform to the form and dimensions shown in Fig. 1.

7.3 *Number of Test Specimens*—Test at least 10 specimens of each type. Discard results if failure occurs between the test fixture and the tissue sample and test additional samples to obtain a total of 10 valid tests. Tissue substrates tend to give higher variances and may require more samples to attain a reasonable estimate of the mean strength.

## 8. Sample Preparation

8.1 *Tissue Preparation:*

8.1.1 Tissue substrate materials should be kept moist at all times with phosphate buffered saline (PBS).

8.1.2 The substrate will be placed face down on gauze soaked in PBS, and the back side will be patted dry with fresh gauze.

8.1.3 The back-side of the tissue sample will be glued to the test fixture using a suitable adhesive. When Mediskin Xenograft is used, the epidermal surface will be glued to the fixture, leaving the dermal surface for test adhesive bonding. Gel-type cyanoacrylate adhesives have been found to be useful for this purpose since they set quickly and adhere to most materials.

8.1.4 After the adhesive has cured (approximately 10 min for cyanoacrylate adhesives), place the fixtures on a cutting board and trim the excess tissue away from the fixture using a sharp scalpel. The scalpel must be held perpendicular to the board to ensure that the tissue sample has the same dimensions as the fixture.

8.1.5 Wrap the tissue with gauze soaked in PBS, place the fixtures in a plastic bag, and place them in a water bath or environmental chamber at 37°C.

8.2 *Preparation of the Adhesive Bond:*

8.2.1 Prepare the test adhesive according to the manufacturer's directions or by other prescribed procedure.

8.2.2 Remove the test fixtures from the plastic bag and pat the surface of the tissue dry with fresh gauze.

8.2.3 Apply sufficient adhesive to uniformly coat the overlap area without significant overflow. Excess adhesive could run over the edge of the substrate, causing artificially high test values. The amount required will have to be determined experimentally. For adhesives that are delivered with a spray device, controlling the amount and distribution of the material will be difficult. It may be necessary to use a template to prevent overspray. Alternatively, petroleum jelly may applied to the portion of the tissue outside of the overlap area to prevent bonding.

8.2.4 Bond the two sides of the test fixture together, taking care to keep the fixtures aligned and to maintain the prescribed overlap.

8.2.5 Apply a force of approximately 1 to 2 N to the bond area until the adhesive sets. For slow-curing adhesives, it may be necessary to use a clamping device that can be left in place while the fixture is returned to the environmental chamber or water bath.

8.3 Measure and record the width and length of the adhesive bond to within 0.05 cm.

8.4 Re-cover the tissue with gauze soaked in PBS, replace the sample in a plastic bag, and return it to the constant temperature environment.

## 9. Test Procedure

9.1 Condition the test specimens for definite periods of time under specified, controlled conditions before testing if desired. For comparative testing, the conditioning time should be 1 h ± 15 min. Recommended conditions for tissue adhesives intended for internal applications are 37 ± 1°C in phosphate buffered saline. For adhesives intended for external topical use, recommended conditions are 30 ± 1°C and 50 ± 5 % relative humidity. For quality control testing with metal or polymer substrates, the recommended conditions are 23 ± 1°C and 50 ± 5 % relative humidity.

9.2 After conditioning, it is recommended that all specimens be stabilized at the test temperature for 15 min before testing if the test temperature is different from the conditioning temperature. Tissue samples must be kept moist throughout the process to prevent shrinkage due to drying. For comparative testing, the test conditions should be 23 ± 1°C and 50 ± 5 % relative humidity (see Annex A1).

9.3 Place the test specimens in the grips of the testing machine so that the applied load coincides with the long axis of the specimen. Load the specimen to failure at a constant cross-head speed of 2 mm/min.

9.4 Record the load at failure (maximum load sustained) and the type of failure (percentage cohesive, adhesive, or substrate failure based on observation of the bond area).

## 10. Calculations

10.1 Calculate the bond area to the nearest 0.01 $cm^2$. Calculate the tensile strength in mega-Pascals (MPa) as the maximum load divided by the bond area.

10.2 Calculate the average and standard deviation for each group of samples.

## 11. Report

11.1 Report the following:

11.1.1 Complete identification of the adhesive tested, including type, source, date manufactured, manufacturer's code number, and lot number.

11.1.2 Complete identification of the substrate used, its thickness, and any method used to clean or prepare the surface prior to bonding. Also report the method used to adhere tissue to the specimen holder.

11.1.3 Estimated amount of adhesive applied.

11.1.4 Method of adhesive application.

11.1.5 Ambient conditions at time of bonding (temperature, humidity, and so forth).

11.1.6 Measured dimensions of the test adherend (length, width, and thickness).

11.1.7 Conditioning of specimen prior to testing.

11.1.8 Maximum, minimum, average, and standard deviation for the tensile strength.

11.1.9 Number of specimens tested.

11.1.10 *Type of Failure*—This should include estimated percentages of cohesive failure in the adhesive, apparent failure in adhesion, and failure in the adherend (substrate) for each specimen.

11.1.11 Test temperature employed.

## 12. Precision and Bias

12.1 A precision and bias statement does not exist for this test method because round robin testing has not yet been performed.

## 13. Keywords

13.1 adhesive bond; tensile strength; tissue adhesive

# ANNEX

**(Mandatory Information)**

## A1. RATIONALE FOR TESTING TEMPERATURE USED FOR COMPARATIVE TESTING

A1.1 As with all mechanical testing, the temperature can have a large effect on the results obtained using this procedure. Ideally, all of the testing would be carried out at the intended use temperature (37°C for internal applications). However, the equipment required for conducting elevated temperature tensile tests is not available in all laboratories. Furthermore, attempting to test samples immediately after removal from the conditioning bath would lead to unacceptable variation in sample temperature at the time of failure. Therefore it was decided to allow the samples to cool to room temperature for 15 to 20 min prior to testing to eliminate that source of variability.

# APPENDIX

**(Nonmandatory Information)**

## X1. PROCEDURE FOR PREPARATION OF FRESH SPLIT THICKNESS PORCINE SKIN GRAFT

NOTE X1.1—The consistency of porcine skin prepared according to this method has not yet been evaluated in comparative testing with commercially available frozen porcine skin. Inconsistencies in preparation are likely to increase the variability of the test results and require a larger number of samples to achieve statistically valid results.

### X1.1 Materials

X1.1.1 Fresh pig skin procured bilaterally from the flanks of the pig.

X1.1.2 Isopropyl alcohol (70 %).

X1.1.3 #20 scalpel blades with handle.

X1.1.4 Dermatome.

X1.1.5 Microtome blades.

X1.1.6 Non-sterile gauze 4×4's.

X1.1.7 Four (4) non-sterile towels.

X1.1.8 Sterile normal saline warmed to 37°C. An antibiotic-antimycotic preparation such as those used for tissue culture should be added to the saline at 10× the recommended concentration.

X1.1.9 Two (2) petri dishes.

X1.1.10 Two (2) cutting boards.

X1.1.11 Needle holder.

X1.1.12 Spray bottle.

X1.1.13 Dermatome cutting board.

X1.1.14 Two (2), 4 penny nails.

X1.1.15 Mineral oil.

X1.1.16 Forceps.

X1.1.17 20 µL micro-pipette.

### X1.2 Method for Preparation

X1.2.1 Fresh, shaved pig skin harvested bilaterally from the flanks of the pig is procured from a local source and transported to the research laboratory in a cooler on ice. Each piece of skin is approximately 6 in. wide and 18 in. long.

X1.2.2 The skin is removed from the cooler and the dermal surface is cleaned with alcohol and gauze, then placed epidermal side up on a cutting board.

X1.2.3 The pig skin is then cut in 4 strips 1.5 in. wide by 18 in. long using a number 20 scalpel blade. The four pieces are covered with a saline soaked surgical towel to inhibit dessication of the tissue.

X1.2.4 A non-sterile surgical towel is placed onto the laboratory countertop. Several gauze 4×4's are placed on top of the surgical towel and then moistened with normal saline using a spray bottle.

X1.2.5 One of the pig skin strips is placed epidermal side down on another cutting board and the underlying fat layer is excised using a microtome blade secured in a needle holder. The fat layer is removed to the level of dermis. Once this task is completed, the skin is immediately placed epidermal side down onto the saline soaked 4×4's and the dermal surface is then covered with 4×4's moistened with normal saline. The gauze 4×4's are covered with a non-sterile surgical towel. The towel is then moistened with normal saline using the spray bottle. The microtome blade is removed and discarded and a new blade is installed. The process is completed for the remaining three pieces of skin.

X1.2.6 Once the fat layer has been removed from each of the strips of pig skin, the cutting board is washed with mild soap and dried.

X1.2.7 One piece of pig skin is placed, dermal side down, on a specifically designed dermatome cutting board. The skin is secured with one nail at each end of the skin.

X1.2.8 A dermatome with a cutting blade set at a cutting depth of 0.13 mm is used to remove the epidermal layer of pig skin. A forceps is used to remove the harvested epidermal layer during the cutting process. Immediately after completing the excision of the epidermis, the nails are removed from the two ends of the skin and the skin is placed back onto the normal saline soaked 4×4's with the freshly harvested dermal side down. The skin is then covered with additional normal saline soaked 4×4's and covered with the normal saline moistened non-sterile surgical towel. The process is completed for the three remaining pieces of skin.

X1.2.9 The non-sterile towel and 4×4's covering the strips of skin are removed and the visible dermal layer of pig skin is wiped with a dry 4×4.

X1.2.10 The prepared skin is cut into strips of the appropriate size for the particular test being conducted.

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