



# Standard Specification for Cover Glasses and Glass Slides for Use in Microscopy<sup>1</sup>

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## 1. Scope

1.1 This specification describes glass covers and slides for use in routine microscopy.

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

## 2. Glass

2.1 *Cover Glass*—Glass shall be colorless, transparent glass, sufficiently free of pits, bubbles, or cloudiness so as not to interfere with end-use performance. Such defects shall be regarded as major defects and limited accordingly. Inspection shall be by the unaided eye in a good light viewing a single cover glass at a time. Refractive index at sodium D line (589.3 nm) shall be  $1.523 \pm 0.005$ .

2.2 *Glass Slides*—Glass slides covered by this specification shall consist of colorless, transparent glass which may have a slight greenish tint when viewed edgewise. It shall be sufficiently free of pits, nicks, bubbles, striae, scratches, and cloudiness when observed by the unaided eye so as not to impair end-use performance. Index of refraction at the sodium D line (589.3 nm) shall be 1.50 to 1.54.

## COVER GLASSES

### 3. Dimensions

3.1 *General Use*—The dimensions of cover glass for general use shall be as follows:

3.1.1 *Square*—22 mm on a side.

3.1.2 *Rectangular*—22 by 30, 22 by 40, 22 by 50, or 22 by 60 mm and 24 by 30, 24 by 40, 24 by 50, and 24 by 60 mm.

3.1.3 *Thickness*—The thickness shall be as follows:

Number	Thickness, mm
1	0.13 to 0.17
1½ <sup>A</sup>	0.16 to 0.19
2	0.17 to 0.25

<sup>A</sup> Specified as optimum by most microscope manufacturers.

3.2 *Limited Use*—The following sizes while recognized, find limited use and are generally not as readily available.

3.2.1 *Square*—18, 24 and 25 mm on a side.

3.2.2 *Rectangular*—24 by 55 mm, 35 by 50 mm, 45 by 50 mm, 20 by 26 mm and 20 by 40 mm.

3.2.3 *Circular*—18, 22 or 25 mm in diameter.

3.2.4 *Thickness*—Thickness Number 0, 0.08 to 0.13 mm.

3.3 *Tolerance*—Tolerance on dimensions other than thickness shall be  $\pm 0.5$  mm.

3.4 *Blood Counting Chambers*—The dimensions of cover glasses for blood counting chambers shall be as follows:

Length, mm <sup>A</sup>	width, mm <sup>A</sup>	Thickness, mm <sup>B</sup>
20	21	0.4 or 0.6
20	26	0.4 or 0.6
25	28	0.4 or 0.6

<sup>A</sup> The tolerance on length and width shall be  $\pm 1.0$  mm.

<sup>B</sup> The tolerance on thickness shall be  $\pm 0.06$  mm.

### 4. Planeness

4.1 *General Use*—Cover glass may be expected to pass the test as described in 14.1.

4.2 *Blood Counting Chambers*—Cover glass shall be plane within 0.002 mm on both sides and shall pass the planeness test as described in 16.2.

### 5. Corrosion Resistance

5.1 While no corrosion tests for cover glasses are hereby required, when desired for critical use, use the test in 14.2.

## GLASS SLIDES

### 6. Dimensions

6.1 *General Use*:

6.1.1 *Size*—75.5 by  $25.5 \pm 0.8$  mm.

6.1.2 *Thickness A*— $1.0 \pm 0.15$  mm.

*Thickness B*— $1.2 \pm 0.15$  mm.

6.2 *Darkfield and Interference Microscopy*:

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6.2.1 *Size*—75.5 by 25.5 ± 0.8 mm.

6.2.2 *Thickness C*—1.20 ± 0.005 mm.

*Thickness D*—0.90 ± 0.075 mm.

*Thickness E*—1.50 ± 0.05 mm.

6.3 *Embryology*—37.5 ± 0.8 or 50.5 ± 0.8 by 75.5 ± 0.8 mm.

6.3.1 *Thickness*—Same as either thickness *A* or thickness *B* in 6.1 on general-use glass slides.

6.4 *Petrography*:

6.4.1 *Size*—25.5 by 45.5, 27 by 46, or 28 by 48 ± 0.8 mm.

6.4.2 *Thickness*—Same as thickness *A* or *B* under general-use slides.

## 7. Planeness and Parallelism

7.1 *General Use*—No requirements.

7.2 *Darkfield, Interference, Embryology, Petrography*—No requirements.

## 8. Corrosion Resistance

8.1 *General Use and Darkfield and Interference Microscopy*—The slides shall not be adversely affected by boiling nor shall they exhibit any pink color in the solubility test.

8.2 *Embryology, Petrography*—No requirements.

## 9. Workmanship

9.1 All edges of all glass slides shall be polished or ground. They shall pass the wettability tests in Section 17. General-use microscope slides shall be smooth and free from nicks or blemishes on the short edge which might impair their serviceability for blood smear work.

9.2 *Frosted End*—One end of one or both surfaces of microscope slides may be frosted to allow marking with a No. 2 pencil or ballpoint pen for a distance of 19 ± 3 mm from the end. Alternatively, both ends of the microscope slide may be frosted for a distance of 9 ± 2 mm.

## GENERAL

## 10. Sampling

10.1 The cover glass and glass slides to be tested for compliance with these specifications shall be selected at random from the lot or shipment. Ten or more cover glasses or slides shall be tested for index of refraction and clarity, resistance to boiling, and solubility.

## 11. Packaging and Package Marking

11.1 *Cover Glass*—General-use cover glass shall be packaged in lint-free boxes generally standing on edge so that the glass is convenient to use without contamination. It should be protected from infiltrating dust and moisture which can cause sticking together of the glass.

11.2 *Glass Slides*—Slides shall generally stand on edge in unit packages to present them conveniently for use by the end user without contamination or loss of cleanliness. Packages shall be sufficiently lint-free to avoid contamination. Unit

packages may be wrapped in a moisture-proof barrier material, either singly or in groups to reduce field contamination, sticking and to extend shelf life.

## TEST METHODS

### 12. Index of Refraction

12.1 Measure with a refractometer and sodium (yellow) light.

### 13. Clarity

13.1 Examine the defects by the unaided eye. This means the use of an emmetropic eye or an ametropic eye corrected to normal vision without further magnification. Examine cover glasses in good light in front of a dark surface. Examine general-use slides with daylight or diffuse uniform artificial light against a uniform dark background. Examine darkfield and interference microscopy slides with a 10× magnifier in a light beam of at least 25 fc against a black background.

### 14. Planeness

14.1 The number of cover glasses specified in Table 1 shall be stacked on a flat surface. The height of the stack shall be measured within ±0.05 mm. A thin stiff aluminum plate cut to the size of the cover glass is to be placed on top of the stack. A weight is placed on top of the plate so that the total mass of the aluminum plate plus the weight is 100 g. Again, the height of the stack is to be measured to within ± 0.05 mm. The difference between the unloaded and loaded readings is the compression. Compression shall not exceed the amount shown in the Table 1.

14.2 Test planeness against an optical flat, using the interferometric method described in National Bureau of Standard Circular 507.

### 15. Thickness Variation

15.1 Permissible thickness variation in any one specimen of cover glass is as follows:

15.1.1 For sizes up to and including 25 mm in any one dimension, not more than 1 % of the specimens measured shall show a greater variation than 0.015 mm in any one specimen.

15.1.2 For sizes above 25 mm in any one dimension, not more than 1 % of the specimens measured shall show a greater variation than 0.020 mm in any one specimen.

### 16. Resistance to Corrosion and Other Defects

16.1 *Resistance to Boiling*—Immerse the slides in distilled water in a closed vessel having a small steam vent and boil continuously for 6 h, occasionally adding boiling water to

**TABLE 1 Quantity of Cover Glasses and Compression Allowed (22 by 22-mm Cover Glass)**

Thickness No.	Quantity of Cover Glass	Compression (Warpage), mm
0	140	2.13
1	100	1.42
1½	86	1.19
2	70	1.01

compensate for evaporation losses. Suspend the slides in water so that they do not come in contact with the containing vessel. The slides shall show no evidence of fogging, chipping, or cracking as a result of the test.

16.2 *Solubility of Cover Glasses*—Clean 24 cover glasses by immersion for 1 min in distilled water, remove, and air-dry for 1 min in a vertical position. Perform this cleaning operation three times, using a new quantity of distilled water for each immersion. Half fill an alkali-resistant 750-mL Erlenmeyer flask with distilled water and boil for 10 min. Allow the water to cool for 3 min and then decant until the volume of the flask is approximately 100 mL. Keeping the flask covered, let it stand until the water has cooled to approximately 70°C. Add 0.2 mL of USP standard phenolphthalein solution and continue to cool to 60°C. Add the cover glasses, one at a time. Superimpose the cover glasses by tilting and gently swirling the flask. Let the solution cool toward room temperature without agitation for 1 h. Absorption of CO<sub>2</sub> from the atmosphere must be avoided during the test. No pink color shall be visible through the 24 superimposed cover glasses.

16.3 *Solubility of Glass Slides*—Clean each individual slide by immersing for 1 min in distilled water, removing, and air drying for 1 min in a vertical position. Perform this cleaning operation three times, using a new quantity of distilled water for each immersion. After the final cleaning operation, place the slides immediately in distilled water containing 0.2 % USP standard phenolphthalein solution which has just been boiled for 10 min in a flask (which in itself will not affect the test solution) and allow to cool (preferably without excessive agitation but with mild stirring with a thermometer) to 60°C. Place the slides in the flask so as to obtain the maximum exposure of area per slide. No pink color shall develop in the test solution at any time during the ensuing 1 h, the solution cooling toward room temperature at that time. Absorption of CO<sub>2</sub> from the atmosphere must be avoided during the 1-h standing.

## 17. Wettability

17.1 *Micro Slides*—To assure that blood or other test material will spread evenly across the surface of the slide, the surface of the slide should not be touched, handled, or fingerprinted on or near the edges and surfaces to be used. The sample slide shall be dipped not more than half way into a beaker of distilled water, and then examined for proper dispersion of the liquid and absence of gaps in the film on either side of the slide.

17.2 *Cover Glass*—If wettability is critical, the following test should be used. Grasp cover glass by edges near one end. Dip bottom half into distilled water. Upon removing, cover glass should show an even coat of water on both sides with no dry spots or beading. If some beading does occur, measure the contact angle between the horizontal plane of the cover glass and the drop of distilled water. Reject cover glass if contact angle is greater than 30°.

## 18. Packaging and Package Marking

18.1 *Product Description*—Packages for both slides and cover glasses should include size, thickness, special information such as selected, frosted end or precleaned, stock number if applicable, quantity or weight, name of manufacturer or distributor, and country of origin. Lot numbers or other manufacturing data shall appear on either the unit or over-packer.

18.2 Packages of slides or cover glasses meeting these specifications may be labeled “Meets ASTM Specification E211, Section \_\_\_\_\_.”

## 19. Keywords

19.1 cover glass; glass; microscopy; slides

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