

D5615

(Rev. 140, Issued: 05-29-15, Effective: 05-29-15, Implementation: 05-29-15)

§493.1274 Standard: Cytology

(b)(1) All gynecologic slide preparations must be stained using a Papanicolaou or modified Papanicolaou staining method.

Interpretive Guidelines §493.1274(b)(1)

The Papanicolaou staining procedure is a polychrome method that enhances differences in cellular morphology. The procedure utilizes a nuclear stain, hematoxylin and two cytoplasmic counterstains, OG-6 and EA. The Papanicolaou method is used for staining cytologic preparations because it provides well-defined nuclear detail, stains cytoplasm of various cell types different colors, and renders transparent cytoplasm. There are a variety of formulas for making hematoxylin, OG-6, and EA stains. The actual staining technique may vary among laboratories depending on the type of stains used and the laboratories' modification of the staining method. Modifications of the staining procedure must include the four main steps of the standard Papanicolaou staining method: fixation, nuclear staining, cytoplasmic staining, and clearing.

Cytology laboratories may receive reagents, solutions, and stains from a manufacturer in large volume stock containers. For ease in handling, portions of these reagents are usually decanted into smaller working containers, which must be labeled in accordance with §493.1252(c). Some manufacturers do not label stain or reagent containers with the expiration date; however, lot numbers and package inserts refer to this information.

If the laboratory uses a manufacturer's kit, the reagents of the kit must not be combined, mixed, or replaced with components of another kit from a different lot number, unless otherwise permitted and specified by the manufacturer in the package insert (use D5419). Laboratories which use automated staining methodologies must follow the manufacturer's instructions (use D5411).

The cytology laboratory must document the expiration date of stock reagents, working stains, and solutions made in the laboratory. Use D5415.

Laboratories may use staining procedures, other than the Papanicolaou method, for staining nongynecologic specimens.

Review the written staining procedure for staining gynecologic specimens. Confirm that the written procedures reflect:

- Stains used (i.e., Harris, Gill or other type of hematoxylin, OG-6, modified OG-6, EA36, EA50, EA65, modified EA) or the identity of a combination counterstain;
- Solutions used (water, alcohol, clearing reagent, acid and bluing agent);
- Concentration of each solution used (i.e., percentage (%) of alcohol, acid, ammonium hydroxide or lithium carbonate solution);
- Length of time or number of dips slides are placed in each stain or solution;
- The staining dishes must be labeled to reflect content (not just lids); and

- Procedure for coverslipping slides.

Current time frames must be specified in the procedure manual for each step in the staining of cytology specimens using the Papanicolaou staining method. Adjustments to time frame changes must be documented.

Step-by-step written procedures must be available and followed to prepare nongynecologic specimens.

Use D5403 if any of the above findings is not met.

The laboratory must ensure that the gynecologic and non-gynecologic stains have been tested to ensure predictable staining characteristics on a daily basis. Use D5473.

NOTE: Any fixatives, reagents, or preservatives intended to be used on one liquid-based manufacturer's instrument must not be used on another manufacturer's instrument.