**D5475**
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## §493.1256 Standard: Control procedures

**(e)(3) Check fluorescent and immunohistochemical stains for positive and negative reactivity each time of use.**

**Interpretive Guidelines §493.1256(e)(3)**

All fluorescent stains, including fluorochrome acid-fast stains, must be tested for positive and negative reactivity each time of use.

**Flow Cytometry**

Staining controls for cell surface immunophenotyping by flow cytometry should consist of either normal, cultured or abnormal cells known to be positive for selected standard antigens and must verify the proper performance of reagents. Frozen or other preserved cells may be used. A negative reagent control must be run for each test cell preparation, and is to consist of monoclonal antibody (ies) of the same species and isotype. Negative reagent controls will consist of:

- For indirect stains, an irrelevant primary antibody, if available, and in all cases, the same secondary antibody(ies) conjugated with the same fluorochrome(s) used in all relevant test combinations; and

- For direct stains, an irrelevant antibody conjugated to the same fluorochrome and at the same fluorochromes: protein ratio used in all relevant test combinations.

**Probes §493.1256(e)(3)**

For flow cell cytometric surface immunophenotyping, is a negative reagent control used to define a threshold for positive staining cells? If not, how does the laboratory define the threshold for positive staining cells?