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Q & A



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Editor:

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Q. I understand that the CAP Laboratory Accreditation Program recently announced that negative controls are no longer required for immunohistochemistry, but I'm still confused. Under what circumstances do I have to run negative controls?

A. The CAP anatomic pathology checklist item concerning the use of negative controls (ANP.22570) was recently revised, effective July 31, 2012, to eliminate the requirement for negative reagent controls in immunohistochemistry so long as the detection chemistry used does not rely on an avidin-biotin linkage. This would include detection products marketed as "polymer," as well as "multimer." Thus, if you are using newer detection reagents, negative reagent controls are no longer required. ANP.22570 discusses two types of negative controls for immunohistochemistry, so a little clarification may be warranted.

The first is the negative *tissue* control, and the second is the negative *reagent* control.

Negative *tissue* controls are those tissue elements in the reaction mixture that are expected to be negative. These negative tissue elements may be an integral part of the patient sample or may be added as "on-slide controls." An example of the former would be lymphocytes in a tonsil being stained with a cytokeratin marker. When negative tissue controls appear to be staining positively, it is an indication of nonspecific reactivity, and appropriate remedial steps should be taken. Both positive and negative *tissue* controls are still required by the CAP and CLIA to assure that stains are working properly.

The negative *reagent* control is a replicate patient tissue slide in which the primary antibody is replaced with either another antibody of the same species and isotype or, in some laboratories, simply diluent. This slide is generally treated with the most aggressive antigen-retrieval protocol used among the various tests in a given case. This control is designed to indicate the presence of nonspecific binding of detection reagents due to the presence of endogenous biotin[€]as may be seen in a variety of normal and neoplastic tissues, most notably liver and kidney.

It is now recognized that the newer "multimer"- and "polymer"-based detection reagents are sufficiently free of background reactivity that a negative reagent control is not generally helpful. The CAP Immunohistochemistry Committee has concluded that the value added by routine use of a negative *reagent* control does not outweigh the cost in terms of labor, reagents and materials, space on staining instruments, and limited available diagnostic tissue. Use of a negative reagent control for all cases is not a CLIA requirement. However, a common-sense approach is required. Negative controls are indicated in cases where there is unexpected staining and should be added to such cases at the discretion of the pathologist.

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Q. Under what conditions would a patient have a normal red blood cell count ($5.25 \times 10^6/\mu\text{L}$) but a low hemoglobin (10.1 g/dL) and a low hematocrit (32.9 percent)?

A. Normal RBC counts may be associated with a low hemoglobin and a low hematocrit in a number of conditions, including thalassemia trait and polycythemia vera (PV) with concurrent iron deficiency anemia. Both conditions are seen with a low MCV. In thalassemia trait, whether alpha or beta thalassemia, there is a structurally abnormal hemoglobin being made. Thus, patients with thalassemia trait have normal to elevated numbers of red blood cells, but, being microcytic and hypochromic, they have a low hemoglobin and a low hematocrit. Interestingly, in polycythemia vera there is an increase in red blood cell production that has escaped normal growth factor controls (that is, erythropoietin independent). In patients with adequate iron and polycythemia vera, this results in increased numbers of normocytic red blood cells and subsequent high hemoglobin and hematocrit. Such patients with polycythemia vera may rapidly deplete their iron stores with an unchecked production of red blood cells. Also in PV, some patients have gastrointestinal blood loss, another factor contributing to their iron deficiency. Thus, red cells from PV patients can become microcytic and hypochromic. While in uncomplicated iron deficiency anemia this results in decreased numbers of RBCs, in polycythemia vera complicated by iron deficiency anemia this may be seen as a normal RBC count, which is actually decreased for the patient.

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