The Laboratory Services Reform Programme

ADVICE NOTE

Use of Tissue Specific Negative Control in Immunohistochemistry

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Clinical Practice Guidance Document Cover Sheet

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Document Owner/Author	Author: Dr Niamh Leonard and Marie Culliton / Professor Martin Cormican
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The Laboratory Services Reform Programme offers the following advice:

1 Advice for Laboratories

1. With respect to Immunohistochemistry (IHC) staining, when undertaking equipment or method verification or when verifying a new antibody clone, non- specific staining in tissues should be assessed as part of the verification. The assessment should include a comprehensive range of tissues representative of tissues likely to be tested.
2. Laboratories should follow the manufacturer's advice with regard to the use of IHC reagents with exceptions based on risk assessment.
3. Laboratories should be conscious of the critical nature of some IHC tests which are used as a "companion diagnostic" that directly influences clinical judgement regarding the likelihood of benefit from specific therapeutic agents.
4. In assessing the risk of deviation from manufacturer's instructions with respect to use of a reagent negative control on each specific tissue sample being examined the key considerations are
    a. the laboratory has established during verification that false positives related to the staining process for that antibody are very unlikely to occur
    b. that experience in use of the antibody continues to support this assessment
    c. that all other controls are functioning appropriately
    d. there is no cause for concern regarding non-specific staining when the Histopathologist is reading and interpreting the stained slides from the specific sample in question
    e. the use of additional material for tissue specific negative controls may result in insufficient remaining material for additional tests that may be essential subsequently to guide patient care
5. If conditions 4a to 4d are met and condition 4e applies the risk of depleting precious tissue is likely to outweigh the risk of omitting a reagent control in most circumstances
6. If conditions 4a to 4d are not met OR condition 4e does not apply including a reagent control is likely to be appropriate unless other risks apply

2 Background

Immunohistochemistry is a technique that uses antibodies to bind to antigens in tissue. The antigen antibody complex is then visualised using a chromogenic or fluorescent detection process before the slide is examined and interpreted by a Histopathologist in the context of other slides and all relevant

clinical information. The detection of antigens and the pattern of distribution in cells and tissues assists in diagnosis and prognosis of disease and may guide clinical judgement regarding likelihood of benefit from specific therapeutic agents. Appropriate controls are necessary to confirm that the observed staining reflects the presence and distribution of the target antigen.

Positive Tissue control: a section from a tissue known to express the antigen of interest that is exposed to the specific antibody followed by the visualisation process A positive result from the positive control indicates that the procedure has functioned as expected in the process performed on that day. This provides a significant measure of assurance that samples in that batch that did not stain do not have the target antigen.

Negative Tissue control: a section from a tissue known not to express the target antigen that is exposed to the specific antibody followed by the visualisation process. A negative result from the negative control indicates that the procedure has not resulted in non-specific staining of tissue that lacks the target antigen on that day. This provides a significant measure of assurance that samples in that batch that did stain do have the target antigen.

Negative Reagent Control: A section of tissue from the case under consideration is incubated with diluent that does not include the specific antibody and is then subjected to the visualisation process. A negative results indicates that the visualisation process has not resulted in staining of tissue independent of the specific antibody to the target antigen. This provides assurance that staining observed on a positive tissue is produced as a result of detection of the target antigen by the specific antibody and is not related to non-specific interaction of the visualisation system with the specimen.

Commentary on Negative Reagent Control

Although manufacturers may recommend a negative reagent control with each tissue sample stained this recommendation must be applied with consideration for the overall clinical risk for the patient. Tissue samples are often of limited size. Formalin fixed paraffin embedded tissue blocks are retained indefinitely. Over the course of a patient's illness it may be necessary to revisit a sample for additional analysis by staining or molecular methods. Tissue blocks are therefore a precious and irreplaceable asset for patient care. The laboratory is the custodian of this precious asset and must at all times act in the patient's interest to ensure that the material is conserved to the greatest possible extent. This requires that the laboratory avoid performing non-essential examinations and that it perform only those examinations that are essential with care to preserve tissue to the greatest possible extent. This document is intended to support laboratories in assessing the relative risk where manufacturer's recommendations for immunohistochemistry reagents recommend a negative reagent control in each case but the consequence of following that recommendation is likely to be depletion of irreplaceable material.

3 References

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Authors: Developed by the Laboratory Services Reform Programme Incorporating The National Clinical Pathology Programme.

Approved By: Martin Cormican MCRN 011105,
HSE Clinical Lead for Laboratory Services Reform Programme.

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