ASVCP quality assurance guidelines: veterinary immunocytochemistry (ICC)

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1. Introduction/Scope

Immunocytochemisty (ICC) is an advanced diagnostic technique that has gained popularity in veterinary medicine, as it allows for relatively inexpensive antigen detection that can be easily correlated with cellular location. The general consensus is that, if applied properly, ICC increases diagnostic accuracy. This document is intended as a resource for clinical pathologists working in a laboratory that performs diagnostic ICC and does not refer directly to flow cytometry. These guidelines are not intended to be all-inclusive; rather, they provide a minimum standard for quality assurance of diagnostic ICC in veterinary settings.

2. Pre-analytical factors important in immunocytochemistry

2.1 Specimen collection, handling, and transport to the laboratory

Air-dried slides and cells in transport media containing saline with 5-10% fetal bovine serum or 10% patient serum are most recommended. Unstained slides can be fixed in neutral buffered formalin or Romanowsky-stained slides may be used if the laboratory performing the test utilizes antigen retrieval methods. Before fixing cells, contact the laboratory to determine the best preservative to use for the antigen of interest. Samples should be transported to the laboratory by a method which will protect against freezing and overheating. Samples should be sent overnight, in protective packaging, with a cold pack for fluids. Acetone permeabilizes cell membranes and inhibits bacterial growth and is a reasonable transport medium only for those samples that will be processed to completion within 24 hours, due to rapid cell lysis in this medium.

2.2 Personnel requirements

Immunocytochemical staining is a specialized test performed in a histology or clinical pathology laboratory. Personnel performing immunocytochemical staining procedures should do so frequently and be familiar with all control tissues and procedures. Veterinary clinical pathologists who interpret the slides should have corresponding Romanowsky stained slides available and should be familiar with the expected staining appearance of controls and tissues for the stains offered by laboratory.

3. Analytical factors important in immunocytochemistry

3.1 Staining method

3.1.1 Staining quality. All slides sent to the pathologist, including patient slides and all control slides, should be examined for consistency and appropriate staining prior to release of the interpretation.

3.1.2 Method validation. Prior to offering a new immunocytochemical stain, the procedure should be tested and optimized until controls stain appropriately without excessive background staining, and consistent results are achieved with replication. As applicable, results should be compared with results achieved using other kits or methods for detecting the same antigen(s). Optimal primary antibody dilution must be determined separately within each laboratory performing ICC. Positive controls should contain both positive and negative areas. Negative patient controls in which the primary antibody is replaced with serum from the same species or an unrelated antibody must be negative. For further information please refer to reference 1.

3.2 Instrumentation and Reagents

3.2.1 Automated stainers. The manufacturer's instructions should be followed for operating and maintaining automated stainers.

3.2.2 Reagents. Manufacturer's recommendations should be followed for storage of all reagents, and reagents should be stored in non-reactive containers. All reagents and antibodies must be labeled with the name of the reagent or antibody, date received, date prepared or opened, expiration date, and name or initials of person who prepared or opened the reagent, as is required for all reagents used in a histology or clinical pathology laboratory.

3.2.3 Controls. Preparation and storage of control slides should follow standard practices with the histology or clinical pathology laboratory. Control slides for immunocytochemistry should be cytology slides and not formalin fixed tissues. Control slides for immunocytochemistry are best used within two weeks if the air-dried preparations are stored at 4°C or longer if placed in a standard freezer or if chemical fixation and antigen retrieval are used. Control slides stored in the freezer should be placed in a plastic container and then in a zip-lock bag containing desiccant.

3.3 Quality assurance/quality control

3.3.1 Follow general recommendations for quality control as detailed in the standard operating procedures (SOP) for the laboratory, including for the automated stainer, if applicable.

3.3.2 Biannual internal quality assurance/quality control. Each laboratory should have a quality system in place to ensure the quality of results. For example, three to five randomly chosen cases should be evaluated by a second pathologist in the laboratory for completeness and accuracy of immunocytochemical interpretation (internal audit).

External QA/QC would be ideal, but is not available through most commercial external quality assurance (EQA) providers.

3.3.3 Procedures manual. Use of SOPs by the laboratory is recommended. Immunocytochemical procedures should, at minimum, follow the recommendations of reagent and equipment manufacturers.

3.3.4 Outsourced procedures. The following materials should be sent to a trusted laboratory performing the desired immunocytochemical reaction: sufficient numbers of unstained cytologic slides (for example, cytocentrifuged preparations with a minimum of 250 cells/μl and a maximum of 500 cells/μl, blood smears, or cytologic smears), the original Romanowsky-stained slide, and the initial cytology report. The submitting laboratory should ensure that the contracted laboratory is in compliance with recommendations in this document.

3.3.5 External quality assessment. Interpretation of ICC reactivity may be compared against immunohistochemistry of similar tissues as long as the antibody clone is the same and has been validated for performance in paraffin-embedded tissues. Similarly, flow cytometry may be used for comparison of results to ICC.

4. Post-analytical factors

Results of immunocytochemical staining, as well as any additional interpretation or explanation, should be linked, in accordance with laboratory policy, to the original cytology report and provided to the client. Testing performed at an external laboratory must be clearly indicated on the report.

5. Acknowledgments

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6. References

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