Correlation Between Different Cytologic Techniques Used on Respiratory Specimens from Racing Thoroughbreds in Southern California

Kathy P. Freeman, DVM, MS, PhD and Wayne Ho, MT (ASCP)

Air-dried, Wright–Giemsa preparations are useful for the rapid evaluation of equine respiratory cytology specimens. Polychrome preparations offer more detail and different information than Wright–Giemsa preparations. Polychrome preparations are more sensitive and accurate than Wright–Giemsa preparations for the identification of complicated processes, mucus character, and patterns associated with obstructive pulmonary disease. Authors’ addresses: 2150 W. 6th Ave., Unit F, Broomfield, CO 80020 (Freeman) and 1050 Prairie Ave., Inglewood, CA 90301 (Ho). © 1997 AAEP.

1. Introduction
Two techniques for cytologic evaluation are currently used by 15 practitioners submitting cytologic specimens from racetracks in southern California (Southern California Equine Foundation). These are (a) a rapid preliminary report available within 1–4 h, using Romanowsky-stained (Wright–Giemsa, or WG) preparations and (b) a detailed report available within 24–48 h, using wet-fixed, polychrome-stained (PC) preparations. The purpose of this investigation is to compare the WG and PC evaluations.

2. Materials and Methods
Respiratory cytology specimens (transtracheal or endoscopic washings or bronchoalveolar lavages) were collected from 157 horses. The method of collection was determined by the individual practitioner and did not affect the results.
Specimens were mixed well and divided for WG and PC techniques. The aliquot for polychrome staining was added immediately to an approximately equal volume of 40–50% ethanol and delivered overnight to a commercial veterinary laboratory for preparation and staining according to previously described methods.1,2 The aliquot for WG preparations was prepared on site and stained as previously described.3 The authors evaluated all 157 specimens to determine the quality of the preparations and for degree of cellularity and types of cells, indicating whether the specimen was satisfactory, unsatisfactory, or marginal for light microscopic interpretation. Adequate cellularity was defined as a moderate number of cells (average of at least 2–5 cells/hpf). Cell types that indicate satisfactory endoscopic or transtracheal washings are alveolar macrophages with columnar or cuboidal epithelial cells representative of alveoli or large or small airways, respectively. Satisfactory bronchoalveolar lavage specimens are defined by the presence of alveolar macrophages. Unsatisfactory specimens are of very low cellularity or
POSTERS

Proceedings of the Annual Convention of the AAEP 1997

Reprinted in the IVIS website with the permission of the AAEP
Close window to return to IVIS

do not contain the requisite cell types. Marginal specimens are of low cellularity (average of <2 cells/hpf).

Seventy randomly selected cases were analyzed separately by each author for correlations between degree (slight or mild, moderate, or marked) and type of inflammation (neutrophilic, lymphocytic, or eosinophilic) and other cell types (epithelial, macrophages, erythrophages, or siderophages), analysis of mucus character, and ability to recognize previously reported cytologic patterns in both WG and PC preparations.

3. Results

A. General Evaluation of Respiratory Cytology Specimens

All 157 of the PC preparations were satisfactory for interpretation. Fifty-nine of 157 PC specimens had little sediment following centrifugation or visual examination, so membrane preparations were made. Ninety-eight of 157 of the WG preparations were considered satisfactory for interpretation. The remaining 59 corresponded to the specimens that received PC membrane preparations; they were of low cellularity in the WG preparations, and confidence in their representativeness and interpretation was low (marginal specimens).

Epithelial morphology was infrequently preserved in WG preparations but was consistently evaluable in PC specimens. Early and aged siderophages were recognized in both WG and PC preparations, but they were more easily detected in PC preparations as a result of distinctive differential staining. Because of the tendency for cells to become very condensed and stain darkly in WG preparations, there were fewer cells that could be definitively identified compared with PC specimens.

B. Detailed Evaluation of Respiratory Cytology Specimens

Seventy specimens were evaluated in detail for correlation between WG and PC techniques and for interobserver variations within techniques. There was interobserver agreement in the identification of cell types in 67/70 specimens in both WG and PC preparations. There was agreement as to degree of neutrophilic and lymphocytic inflammation in 44/70 of the cases. In the remaining 26 cases, there were only 2/70 in which there was disagreement regarding degree of neutrophilic or lymphocytic inflammation that differed by more than one degree (e.g., mild or marked instead of mild or moderate or moderate or marked).

In 13/70 cases in which eosinophils were identified, there were seven cases with eosinophilic inflammation in PC preparations in which eosinophils were not detected in WG preparations. All of these cases required membrane preparations for PC preparations; they had WG specimens of low cellularity that were considered marginal for interpretation.

Patterns identified in PC preparations were correctly identified in 35/70 WG preparations. Uncomplicated patterns other than obstructive pulmonary disease were underdiagnosed in 26 cases in WG compared with PC preparations. Obstructive pulmonary disease or complicated patterns indicative of multiple processes were underdiagnosed in WG preparations. They were detected in only 1/28 WG preparations in which these conditions were identified in corresponding PC preparations.

The inability to accurately assess mucus character and casts typical of cytologic obstructive pulmonary disease and the evaluation of epithelial morphology presented the most significant problem in pattern identification. The inability to accurately identify increased mucus thickness and cytologic obstruction accounted for 24/35 of the mismatches between WG and PC preparations.

4. Discussion

The results of this study indicate that satisfactory specimens were more often obtained in PC than WG preparations. The availability of membrane concentration techniques for PC preparations enhanced the validity of interpretations of cytologic specimens of low cellularity that are marginal by using WG techniques. There was good correlation between WG and PC techniques for the evaluation of cell types and degree of inflammation. However, cytologic patterns identified in PC preparations were correctly identified in only 50% of the corresponding WG preparations. This was attributed primarily to the inability to analyze mucus character and casts that support increased mucus thickness and cytologic obstruction of airways.

The recognition of patterns and the identification of uncomplicated processes (single pattern) versus complicated processes (multiple patterns) were considered by the participating practitioners to be useful information. The rapid availability of the WG results was of benefit in confirming pulmonary inflammation and in determining the type of inflammation and whether siderophages or erythrophages were present. This provided rapid feedback for the veterinarian and helped in the formulation of initial treatment plans.

The results of this study confirm that the PC preparations are more sensitive and specific in the detection of patterns and the differentiation of uncomplicated and complicated conditions. The PC evaluation provided a more detailed clinicopathologic analysis for the submitting veterinarian. These findings were used to further refine patient care, treatment, and monitoring plans and were used for client education regarding the etiopathogenesis and prognosis and treatment of equine respiratory disease.

The authors thank the Southern California Equine Foundation Dolly Green Research Laboratory for support for this project. We greatly appreciate the efforts of all participating veterinarians and their input regarding this paper.

References