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Ocular Angiosarcoma in the Horse: Morphological and Immunohistochemical Studies

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Abstract. Angiosarcomas arising in ocular tissues of four aging horses are described. Tumors were locally invasive and eventually metastasized via the mandibular and cervical lymph nodes. Pathologically, the tumors contained well-differentiated regions, in which vascular channels were lined by pleomorphic endothelial cells, as well as poorly-differentiated regions, in which vascular channels were either rudimentary or absent. Red blood cells were scarce in vascular structures formed by the tumors. Factor VIII related antigen (VIII:RAg), a blood vascular endothelial marker, was demonstrated by immunohistochemistry in tumor cells in both the well-differentiated and poorly-differentiated regions. The results suggest that ocular tumors arose in blood vascular endothelium and represented true hemangiosarcomas although a lymphatic endothelial origin could not be excluded. Immunohistochemical demonstration of VIII:RAg is a valuable adjunct to conventional stains in the diagnosis of equine vascular neoplasia.

Malignant vascular tumors are classified as either hemangiosarcoma or lymphangiosarcoma when the cell of origin is known, and angiosarcoma when the cell of origin is uncertain.[14,15] Factor VIII related antigen (VIII:RAg) is synthesized by endothelial cells of blood vessels, and is detectable within megakaryocytes and platelets.[7] Lymphatic endothelial cells probably synthesize less VIII:RAg than do blood vascular endothelial cells although this issue is currently the subject of controversy.[2,4,12,16] Factor VIII:RAg has been used as a discriminatory immunohistochemical marker of blood vascular endothelium in human tumors.[4,12,16]

Malignant vascular neoplasia is uncommonly described in the horse. Only two cases of ocular angiosarcoma have been reported.[9,11] This paper reports the salient morphological features of ocular angiosarcoma in the horse. The results of immunohistochemical staining for VIII:RAg was initially performed to assist in the determination of the cell of origin since vascular channels in tumors were largely devoid of blood and may have originated in lymphatic endothelium. Equine ocular angiosarcoma had a characteristic clinical presentation, (Hacker DV, Moore PF, Buyukmihci NC: submitted, J Am Vet Med Assoc, 1985) and all tumors strongly expressed VIII:RAg suggesting that they probably arose in blood vascular endothelium.

Materials and Methods

Tissues excised at biopsy or necropsy were fixed in 10% neutral-buffered formalin. Globes were fixed in either Zenker's acetic acid or Bouin's fixative. They were processed routinely for light microscopy and stained with hematoxylin and eosin (HE). Paraffin sections from each case were subjected to immunohistochemical procedures to demonstrate VIII:RAg. Hemangiosarcomas of non-orbital tissues and normal lymphoid tissues from other horses were also stained for VIII:RAg. Sections were deparaffinized and treated with 0.1% trypsin (Sigma Chemical Company, St. Louis, MO) and 0.1% $CaCl_2$ in 0.02 M Tris buffered saline (pH 7.8) for 20–40 minutes at 38 C. Sections were blocked with 1% bovine serum albumin for 30 minutes, and a 1:1,000 dilution of rabbit antiserum to human VIII:RAg (Dako Corporation, Santa Barbara, CA) was then applied for 18–24 hours at 4 C. A 1:200 dilution of biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) was next applied to the sections for 30 minutes at 25 C. A 1:100 dilution of an avidin biotinylated-horseradish peroxidase complex (Vector Laboratories) was then applied for 30 minutes at 25 C. The disclosing reaction was performed in a peroxidase substrate solution which consisted of 0.05% diaminobenzidine tetrahydrochloride, 0.04% $NiCl_2$ and 0.01% H_2O_2 in 0.1 M Tris-HCl which was filtered and adjusted to pH 7.6. Sections were lightly counterstained with hematoxylin. A complete set of negative and positive control sections was included in each run. Appropriately diluted normal rabbit serum was substituted for the primary antiserum in the negative control sections.

Specificity of the commercial anti-human VIII:RAg was investigated by substituting a highly specific rabbit anti-human VIII:RAg (Janet Harrison, UC Medical Center, Sacramento, CA) which had previously been absorbed with serum from a patient with von Willebrand's disease who lacked VIII:RAg. This antiserum was then used to stain paraffin-embedded sections of equine ocular tumors and sections of fresh frozen normal equine mesenteric lymph node.

Table 1. Clinical summary of equine ocular angiosarcoma cases.

Horse	Breed	Sex	Age (yr)	Location of Primary Tumor	Clinical Course
1	Thoroughbred	MC	9	Left lateral perilimbal conjunctiva	Left eye enucleation; radiation therapy; tumor recurrence in left orbit; subsequent mandibular lymphadenopathy and palpable masses in tracheal and pectoral regions; died (colic).
2	Appaloosa	F	15	Left dorso-medial perilimbal conjunctiva	Left orbital exenteration; radiation therapy; tumor recurrence in left orbit; developed forelimb edema; euthanized.
3	Quarter horse	MC	13	Ventro-medial conjunctival fornix	Radiation therapy; new mass appeared behind left ear simultaneously with forelimb edema; euthanized.
4	Arab	MC	17	Left palpebral conjunctiva	Treatment not attempted; tumor invaded left side of face; mandibular and retropharyngeal lymphadenopathy; progressive deteriorating course prompted euthanasia.

MC = castrated male.

Results

The tumors occurred in aging horses and originated in the conjunctiva at various sites often invading the cornea (Table 1). They appeared as poorly circumscribed, firm, edematous, thickened regions in the affected ocular tissues and lacked substantial vascularity. The tumors were quite invasive locally and recurred after surgical extirpation and radiation therapy. Evidence for lymphatic metastasis was present in all horses and prompted euthanasia of three horses. The entire clinical course from initial presentation to death occupied a mean of 12 months (range: 6–18 months).

Additional gross lesions were present in horse 3 and horse 4 which were necropsied. An 8 × 10 × 10 cm firm pale mass was seen in the left temporalis muscle, and a 10 × 10 × 1 cm yellow mass, containing red-black foci, was seen in the cranial mediastinum of horse 3. Firm pale tissue, containing dark red foci, had invaded the left eyelid and conjunctiva and had extended dorsally behind the limbus as well as throughout the entire left side of the face into the submandibular region of horse 4. Invasion of the mandibular lymph nodes and the entire left cervical lymph node chain was seen in both horses.

The microscope architecture of the tumors varied considerably from area to area within the same tumor. Two main patterns of growth were recognized to which the designations of capillary and solid were applied. The capillary pattern was characterized by the presence of a network of well-developed vascular channels lined by plump, hyperchromatic, pleomorphic endothelial cells. A scant collagenous stroma separated the channels, and circulating red blood cells were usually absent (Fig. 1). The solid pattern was composed of sheets of pleomorphic cells with hyperchromatic, large oval nuclei and poorly defined cytoplasmic borders (Fig. 2). Rudimentary vascular channels in the form of clefts were often present between small bundles of these cells (Fig. 3). Tumor giant cells containing one or several nuclei were occasionally seen within particularly undifferentiated regions. Mitotic activity was frequently seen in the neoplastic cells in both patterns of tumor growth. The solid growth pattern usually incited a marked scirrhous response which blended with the neoplastic cells and made delineation of the tumor boundaries difficult (Fig. 4). An intense lymphocytic, plasmacytic inflammatory infiltrate was frequently seen within the tumor tissue and at the borders (Fig. 3). In sections consisting almost entirely of the solid growth pattern, the inflammatory infiltrate and the scirrhous response to the tumor made recognition of the underlying neoplastic nature of the process difficult. In fact, earlier biopsies in horses 2 and 3 were diagnosed as cellulitis by pathologists who were unaware of the existence of ocular angiosarcomas in the horse.

Preliminary studies, in which non-specific binding of the immunoglobulin reagents was blocked in the customary way with 10% serum from the species in which the secondary antibody was raised, were uniformly unsuccessful and only resulted in high background staining; specific endothelial VIII:RAg localization was not seen. It was reasoned that the use of blocking serum may have led to absorption of anti-VIII:RAg antibodies from the primary antiserum by residual cross-reactive VIII:RAg in the blocking serum. The substitution of bovine serum albumin for goat serum in this step provided the solution to the problem and enabled good localization of VIII:RAg with low background staining. Brief trypsin digestion of the sections was also necessary to achieve satisfactory staining.

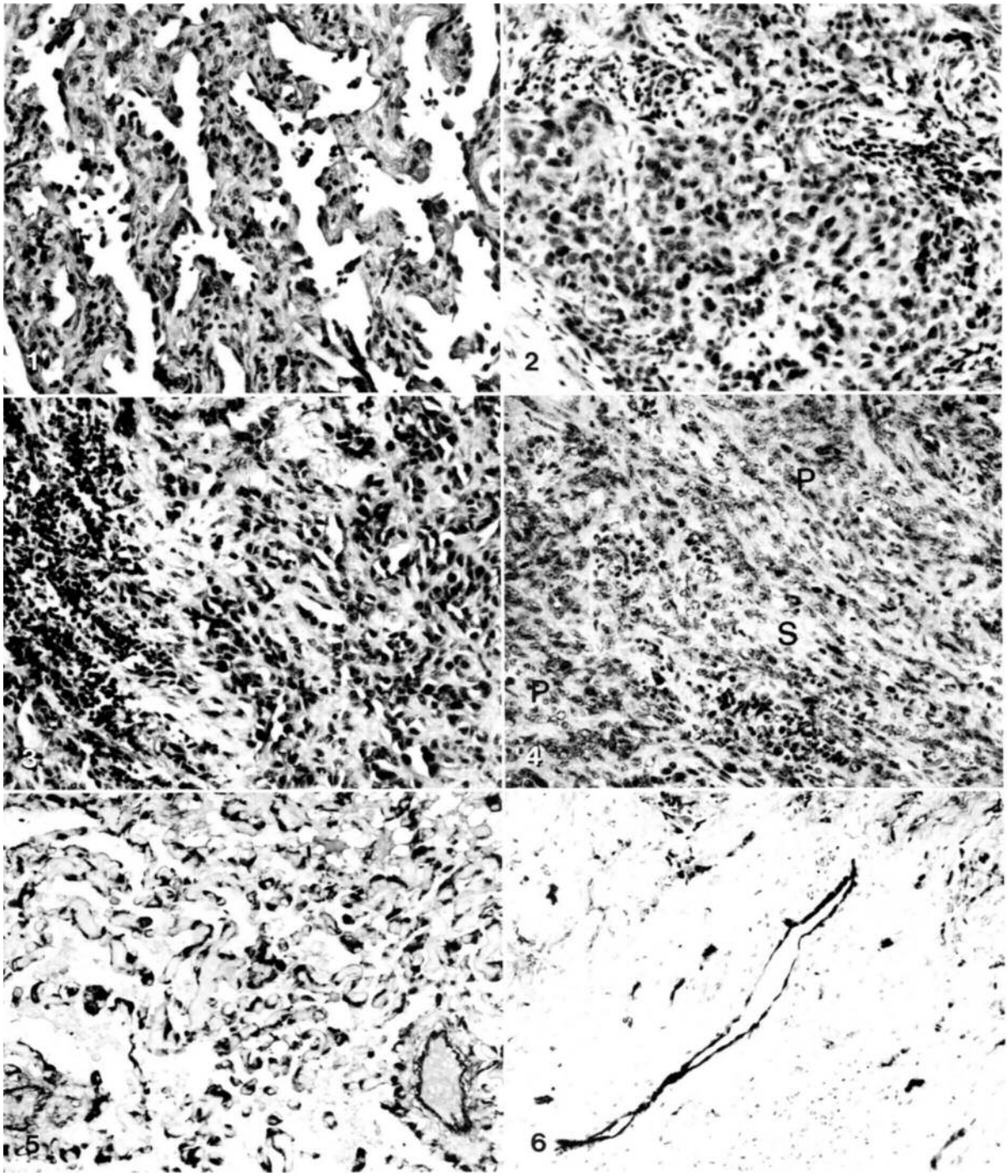

Fig. 1. Capillary growth pattern in ocular angiosarcoma. HE.

Fig. 2. Solid growth pattern; cells are pleomorphic, hyperchromatic, and have poorly-defined borders. Vascular spaces are not apparent. HE.

Fig. 3. Rudimentary vascular channels in an otherwise solid region of angiosarcoma. A marked lymphocytic, plasmacytic infiltrate demarcates the extent of the tumor. HE.

Fig. 4. Poorly differentiated solid regions of angiosarcoma (P) merging with the stroma (S). HE.

Fig. 5. Factor VIII:RAg immunoreactivity (black precipitate) in cells lining vascular channels in capillary regions of angiosarcoma.

Fig. 6. Factor VIII:RAg immunoreactivity in the endothelial cells of a normal vessel adjacent to angiosarcoma.

Endothelial cells lining blood vessels in normal equine tissues stained intensely for VIII:RAg using the revised methodology. Neoplastic endothelial cells lining the vascular channels in sections of hemangiosarcoma from non-ocular tissues of other horses stained positively for VIII:RAg. The vascular channels in these tumors contained circulating red blood cells, and hence were likely to have originated in blood vascular endothelium. The vast majority of endothelial lining cells of normal lymph node sinuses and lymphatics were devoid of VIII:RAg although an occasional lymphatic endothelial cell exhibited positive staining. Staining of sections of fresh frozen equine lymph node for VIII:RAg revealed a much higher frequency of staining of endothelial cells lining sinuses and lymphatics. However, blood vascular endothelium in the same sections was more uniformly and intensely stained. Similar results were obtained with the commercial and highly specific antisera to VIII:RAg. These results established that VIII:RAg was a good marker for normal and neoplastic blood vascular endothelium in the horse. However, VIII:RAg staining alone could not unequivocally discriminate between blood vascular and lymphatic endothelium in normal tissues.

Attention was next directed at the ocular tumors. The cells lining vascular channels in the capillary regions of the ocular angiosarcomas were intensely stained for VIII:RAg (Fig. 5). The intensity of staining closely matched that present in the endothelium of normal vessels in the tumors (Fig. 6). Staining was also seen in the less differentiated solid regions regardless of the presence or absence of rudimentary vascular channels or clefts. However, in this situation the staining was usually less intense and less consistent (Fig. 7). Similar results were obtained with the highly specific antiserum to VIII:RAg.

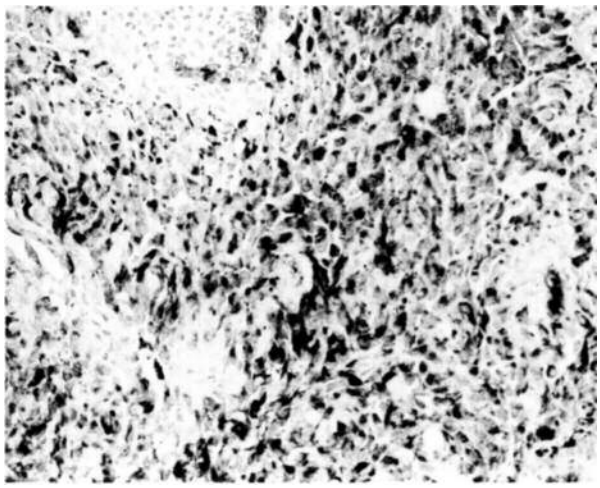

Fig. 7. Solid region in angiosarcoma. Factor VIII:RAg immunoreactivity present in many cells, weak in some.

Discussion

Equine ocular angiosarcoma is a newly recognized syndrome with a characteristic clinical and pathological presentation. The tumors arose in the conjunctiva of aging horses, were locally invasive, and eventually metastasized. Pathologically, tumors were most easily recognized when they formed endothelial-lined channels which usually lacked red blood cells. Less-differentiated, solid areas were visible in all sections, and in some sections they predominated. Recognition of the vascular nature of the tumors was not always possible in these regions. Factor VIII:RAg was readily demonstrated in all tumors in both the well-differentiated and poorly-differentiated regions. Factor VIII:RAg was strongly expressed by normal and neoplastic equine blood vascular endothelium in other tissues and less intensely by lymphatic endothelium. Hence, it is likely that the ocular angiosarcomas originated in blood vascular endothelium and are probably hemangiosarcomas.

Factor VIII:RAg is a well-characterized marker of blood vascular endothelium in man.[7] Controversy exists in regard to VIII:RAg expression by lymphatic endothelium.[2,4,12,16] This situation appears to have some basis in interlaboratory differences in staining technique. Recently, it was suggested that Kaposi's sarcoma originated in lymphatic endothelium based on negative staining for VIII:RAg and other markers, particularly ATPase, alkaline phosphatase, and DR antigen, which were normally expressed by blood vascular endothelium and not by lymphatic endothelium.[2] In that study normal lymphatic endothelial cells rarely expressed VIII:RAg. The results were obtained with frozen sections and trypsin-digested plastic sections and would appear to be immune to the potential spurious staining reactions observed on occasion in paraffin sections.[19] Our results in the horse suggest that both normal blood vascular and lymphatic endothelium synthesize VIII:RAg although lymphatic endothelial expression of VIII:RAg is less consistent and intense and is most reliably detected in frozen sections. Lymphatic and blood vascular endothelium are believed to have a common embryological origin,[10] hence it is not surprising that both express VIII:RAg. Indeed, the application of multiple discriminatory markers would be necessary to conclusively establish the cell of origin in the equine tumors. Many of these markers such as DR, alkaline phosphatase and ATPase[2] do not survive the rigors of conventional processing and paraffin embedding and were not evaluated in these tumors which therefore were designated angiosarcomas.

The discovery that poorly-differentiated regions in the equine tumors expressed VIII:RAg is interesting and unexpected based on previous results in man in

which poorly-differentiated regions of hemangiosarcoma often did not express VIII:RAg.[4,16] It would appear that VIII:RAg is a valuable marker of blood vascular endothelial differentiation in the horse and that VIII:RAg staining is of considerable value in identifying poorly-differentiated vascular tumors which lack endothelial-lined vascular channels.

Vascular neoplasia is exceedingly uncommon among tumors of the eye and orbit in the horse.[3,8,9,11,18] Only three cases of vascular neoplasia were encountered,[9,11,18] two of which were hemangiosarcomas of the palprebral conjunctiva.[9,11] The skin has the highest incidence of neoplasia of any organ system in the horse.[13] In a recent survey of cutaneous vascular neoplasia, only three hemangiosarcomas were described and none involved the skin of the face.[6] In more general surveys of neoplasia in horses, vascular tumors accounted for less than 1% of the tumors.[1,17] Hence it would appear that malignant vascular neoplasia, regardless of site, is uncommon in the horse, and our four cases of ocular angiosarcoma appear to be almost without precedent.

In man, the most common form of angiosarcoma primarily affects the elderly and involves the face and scalp.[15] These tumors have a poor prognosis and frequently metastasize to the cervical lymph nodes. Trauma, solar irradiation, and X-irradiation have been suggested as etiological factors. The importance of solar irradiation has been downplayed due to the high prevalence of tumors in haired regions. A high incidence of vascular neoplasia, including hemangiosarcoma, was reported in the perilimbal conjuctivae of beagle dogs housed outdoors at 1,500 meters elevation in a colony in Colorado.[5] Solar irradiation was considered a likely etiological factor in these cases due to the altitude and exposed housing conditions. The conjunctiva does not have the hair and pigment protection afforded skin and would seem to be more susceptible to the ravages of ultraviolet irradiation. The horses with ocular angiosarcoma originated in California, an environment which experiences intense solar irradiation in summer, and conceivably, ultraviolet irradiation is a likely etiological consideration in these cases also.

Ocular angiosarcoma should be considered in the differential diagnosis in cases of orbital cellulitis and spindle cell variant of squamous cell carcinoma. Careful evaluation of multiple sections should enable an accurate diagnosis in most cases. Factor VIII:RAg is a good immunohistochemical marker for normal and neoplastic blood vascular endothelium in the horse and should serve as a valuable adjunct to conventional morphological evaluation in cases presenting diagnostic difficulty.

References

1 Baker JR, Leyland A: Histological survey of tumors of the horse, with particular reference to those of the skin. Vet Rec **96:**419, 1975

2 Beckstead JH, Wood GS, Fletcher V: Evidence for the origin of Kaposi's sarcoma from lymphatic endothelium. Am J Pathol **119:**294, 1985

3 Blodi FC, Ramsey FK: Ocular tumors in domestic animals. Am J Ophthal **64:**627, 1967

4 Guarda LA, Ordonez NG, Smith JL Jr, Hansen G: Immunoperoxidase localization of factor VIII in angiosarcomas. Arch Pathol Lab Med **106:**515, 1982

5 Hargis AM, Lee AC, Thomassen RW: Tumor and tumor-like lesions of the perilimbal conjunctiva in laboratory dogs. J Am Vet Med Assoc **173:**1185, 1978

6 Hargis AM, McElwain TF: Vascular neoplasia in the skin of horses. J Am Vet Med Assoc **184:**1121, 1984

7 Jaffe EA: Synthesis of factor VIII by endothelial cells. *In:* Biology of Endothelial Cells, ed. Jaffe EA, p. 209. Martinus Nijhoff Publishers, Boston, 1984

8 Kircher CH, Garner FM, Robinson FR: Tumors of the eye and adnexa. Bull WHO **50:**135, 1974

9 Lavach JD, Severin GA: Neoplasia of the equine eye, andexa, and orbit: a review of 68 cases. J Am Vet Med Assoc **170:**202, 1977

10 McClure CFW: The endothelial problem. Anat Rec **22:** 219, 1921

11 Morgan G: Ocular tumors in animals. J Small Anim Pract **10:**563, 1969

12 Mukai K, Rosai J, Burgdorf WHC: Localization of factor VIII-related antigen in vascular endothelial cells using an immunoperoxidase method. Am J Surg Pathol **4:**273, 1980

13 Priester WA, Mantel N: Occurrence of tumors in domestic animals: data from 12 United States and Canadian colleges of veterinary medicine. J Natl Cancer Inst **47:** 1333, 1971

14 Robinson WF, Maxie MG: The cardiovascular system. *In:* Pathology of Domestic Animals, Jubb KVF, Kennedy PC, and Palmer N, p. 67. Academic Press, Inc., New York, 1985

15 Rosai J, Sumner HW, Kostianovsky M, Perez-Mesa C: Angiosarcoma of the skin: a clinicopathologic and fine structural study. Hum Pathol **7:**83, 1976

16 Sehested M, Hou-Jensen K: Factor VIII related antigen as an endothelial cell marker in benign and malignant diseases. Virchows Arch [Pathol Anat] **391:**217, 1981

17 Sundberg JP, Burnstein T, Page EH, Kirkham WW, Robinson FR: Neoplasms of equidae. J Am Vet Med Assoc **170:**150, 1977

18 Vestre WA, Turner TA, Carlton WW: Conjunctival hemangioma in a horse. J Am Vet Med Assoc **180:**1481, 1982

19 Wilson AJ: Factor VIII-related antigen staining by immunoperoxidase in smaller laboratories: a potential problem. Am J Clin Pathol **81:**117, 1984

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