Biochemical Basis for the Difference Between Normal and Atherosclerotic Arterial Fluorescence

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The observation that laser-induced fluorescence (LIF) spectra of atherosclerotic and normal artery are different has been proposed as the basis for guiding a “smart” laser angioplasty system. The purpose of this study was to investigate the causes of this difference in LIF. Helium-cadmium laser-induced (325 nm) fluorescence was recorded from pure samples of known constituents of normal and atherosclerotic artery including collagen, elastin, calcium, cholesterol, and glycosaminoglycans. Similarities between the LIF spectra of atherosclerotic plaque and collagen and normal aorta and elastin were noted. LIF spectroscopy was then performed on specimens of atherosclerotic aortic plaque (n=9) and normal aorta (n=13) and on their extracted lipid, collagen, and elastin.

Lipid extraction did not significantly alter atherosclerotic plaque or normal aortic LIF, suggesting a minor contribution of lipid to arterial LIF. The LIF spectra of normal aorta wall was similar to the spectra of the extracted elastin, whereas the LIF spectra of atherosclerotic aortic plaque was similar to the spectra of the extracted collagen. These observations are consistent with the reported relative collagen-to-elastin content ratio of 0.5 for normal arterial wall and 7.3 for atherosclerotic plaque. A classification algorithm was developed to discriminate normal and atherosclerotic aortic spectra based on an elastin and collagen spectral decomposition. A discriminant score was formed by the difference of elastin and collagen (E−C) coefficients and used to classify 182 aortic fluorescence spectra. The mean E−C value was +0.83±0.04 for normal and −0.48±0.07 for atherosclerotic aorta (p<0.001). Classification accuracy was 92%. With 325-nm excitation, collagen and elastin are therefore the major fluorophores of aortic atherosclerotic plaque and normal aortic wall, respectively, and the difference between normal and atherosclerotic arterial fluorescence appears to be due to differences in relative collagen and elastin content. Consistent with this observation, a classification algorithm based on a collagen and elastin spectral decomposition can accurately classify normal and atherosclerotic aortic fluorescence spectra. Other laser lines may excite different chromophores. These findings will require validation for muscular arteries. (Circulation 1989;80:1893–1901)

The difference between the laser-induced fluorescence spectra of atherosclerotic plaque and normal arterial wall has been well established1–5 and has been proposed as the basis for developing a “smart” laser angioplasty system. This system would incorporate fluorescence spectroscopic feedback to guide high-power laser energy so that only tissue with the fluorescence characteristics of atherosclerotic plaque would be ablated. Arterial tissue fluorescence has been induced with ultraviolet1,3 and visible laser irradiation2,4,5 and several classification algorithms have been evaluated for discriminating normal and atherosclerotic arterial tissue.1–6 In these reports, normal and atherosclerotic arterial fluorescence differed, but the cause of this fluorescence difference was not investigated. Fluorescent substances have been extracted from atherosclerotic plaque,7–11 but it is unclear whether these are the fluorophores responsible for the observed differences in the fluorescence of intact normal and atherosclerotic artery.
The ability of fluorescence spectroscopy to guide selective plaque ablation without vessel perforation will depend on the accuracy of arterial tissue-type classification. Classification schemes that have been developed to discriminate normal and atherosclerotic fluorescence rely on absolute fluorescence intensity, the ratio of fluorescence intensity at two wavelengths, fluorescence peak location, and several linear and nonlinear pattern recognition algorithms. These classification schemes were derived from an examination of spectral patterns and not from an understanding of the nature of arterial fluorophores. It may be anticipated that classification algorithms based on arterial fluorophores would not only accurately classify arterial tissue but would also provide additional information concerning arterial composition. Accordingly, the purpose of this study was to investigate the cause of the difference between normal and atherosclerotic aortic fluorescence induced by 325-nm light and to then use this information to develop a spectral classification algorithm.

**Methods**

This study used several experimental protocols to investigate the basis for the difference between normal and atherosclerotic arterial fluorescence. First, laser-induced fluorescence spectra were recorded from purified laboratory samples of known constituents of atherosclerotic plaque and normal arterial wall for comparison with intact arterial fluorescence. Second, because lipid, collagen, and elastin account for well over half of the dry weight of the arterial wall, these constituents were extracted from normal and atherosclerotic aorta and were analyzed by laser-induced fluorescence spectroscopy to assess whether there were unique spectroscopic features of these compounds in normal or atherosclerotic artery. Third, fluorescence microscopy of normal and atherosclerotic arterial tissue was performed to identify the major fluorescent structures in the vessel wall. Last, a spectral classification algorithm was developed to discriminate normal and atherosclerotic aortic spectra and was evaluated for classification accuracy.

**Helium-Cadmium Laser**

For all spectroscopy, a continuous wave helium-cadmium laser (model 356-5M, Omnicrome, Chino, California) operating at a wavelength of 325 nm and output power of 5–10 mW was coupled to a 400-μm optical fiber (Diaguide, Fort Lee, New Jersey) and was used to induce tissue or compound fluorescence. The experimental apparatus is shown in Figure 1. A custom beamsplitter enabled transmission of the exciting laser irradiation and collection of tissue fluorescence using the same fiber. A shutter (Vincent Associates, Rochester, New York) was positioned in the optical path to limit laser irradiation to the approximate period of fluorescence sampling (500 msec), thereby minimizing tissue photobleaching. The distal end of the optical fiber was positioned in contact with the tissue or compound surface. Fluences used for fluorescence excitation ranged from 4 to 12 mJ/mm². The resulting laser-induced fluorescence was focused by an achromatic lens and imaged onto the slit of a spectrograph (Monospec 27, Jarrell-Ash, Waltham, Massachusetts). An ultraviolet block filter prevented reflected helium-cadmium laser irradiation from entering the spectrograph. Fluorescence was spectrally dispersed (grating 150 grooves/mm, blaze 450 nm) along the horizontal axis of the exit plane of the spectrograph and imaged onto a 700-element intensified linear diode array detector (model ST-100, Princeton Instruments, Princeton, New Jersey) and trans-
ferred (by a GPIB interface, National Instruments, Austin, Texas) to a microcomputer (IBM PC AT) for storage and analysis. To improve the signal-to-noise ratio, 10 fluorescence spectra were recorded and averaged from each site. Individual spectrum acquisition time was 33 msec. A background fluorescence or baseline signal was subtracted from each tissue spectrum to correct for nonzero background caused by dark noise in the linear diode array and to correct for the numerical offset inherent in the analog-to-digital conversion. Fluorescence intensity at each wavelength was measured in arbitrary units based on the amplitude of the signal detected by the linear diode array. Each spectrum was spectrally and radiometrically calibrated using a mercury vapor lamp (UVP, San Gabriel, California) and an NBS-traceable calibrated white light source (model 245A, Optronics, Orlando, Florida), respectively. Radiometric calibration was used to correct the nonuniformities in spectral response inherent in the fluorimetry instrumentation.

To facilitate comparison of spectral similarities, displayed spectra were normalized to peak intensity. By normalization, dependence on procedural variables affecting light collection (e.g., surface topology, fiber-to-tissue orientation, and on excitation and detection efficiency) was eliminated, and individual spectra could be compared.

**Laser-Induced Fluorescence Spectroscopy of Known Constituents of Normal and Atherosclerotic Arterial Tissue**

Laser-induced fluorescence spectra were recorded from commercially purified laboratory samples of known constituents of atherosclerotic plaque and normal artery to determine which constituents had fluorescence emissions after ultraviolet excitation and to compare the fluorescence spectra of the individual constituents with the fluorescence of intact atherosclerotic and normal artery. Spectra were recorded from Type I and III bovine collagens (Sigma Chemical, St. Louis, Missouri), human aortic elastin (Elastin Products, Pacific, Missouri), bovine elastin, calcium phosphate, calcium hydroxyapatite, cholesterol, and glycosaminoglycans (chondroitin sulfate, hyaluronic acid, and heparan sulfate) (Sigma Chemical). All reagents were in solid powdered form except cholesterol, which was dissolved in isopropyl alcohol.

**Lipid, Collagen, and Elastin Extraction From Normal and Atherosclerotic Aorta**

In view of their major contribution to normal and atherosclerotic arterial tissue content, lipid, collagen, and elastin were serially extracted from normal and atherosclerotic aorta, and fluorescence spectra were recorded from these constituents. Extraction was performed on 13 specimens of fresh cadaveric human normal aorta (from seven cadavers) and on nine specimens of thick atherosclerotic aortic plaque (from eight cadavers). Approximately 1.5×1.5-cm specimens were isolated for analysis. Normal specimens were analyzed after removal of the adventitia. Atherosclerotic plaque specimens were analyzed after scalpel resection of the plaque from the underlying aortic media. Histologic analysis of adjacent segments was performed to confirm tissue structure. Laser-induced fluorescence spectra were recorded initially from the intact specimens and again after homogenization of the specimens in a Polytron homogenizer. Lipid extraction was then performed from the homogenized specimens by successive suspension in acetone for 18 hours and ether for 18 hours. Spectra were recorded from the extracted lipid and from the defatted specimens. Collagen and elastin were isolated by heat extraction by the method of Neuman and Logan. The collagen was solubilized by heat autoclaving at 121°C and 1 atmosphere gauge pressure for 2 hours. Centrifugation at 15,000 rpm for 20 minutes resulted in an insoluble pellet or residue containing elastin and a collagen rich supernatant. Fluorescence spectra were recorded from the supernatant. An additional 10 hours of autoclaving of the pellet was performed to fully solubilize any residual non-elastin protein. After centrifugation, the elastin rich pellet was rinsed, and fluorescence spectra were recorded. Fluorescence spectra were not analyzed from the final supernatant because prolonged heating altered collagen fluorescence (L. Laifer, personal observation). Amino acid analysis was performed on the 2-hour supernatants and 12-hour residues of two normal and three atherosclerotic specimens to confirm the collagen and elastin content, respectively. Collagen content was calculated based on hydroxyproline content using reagent bovine collagen (Sigma Chemical) as a standard. Elastin content was determined based on the number of residues of desmosine and isodesmosine (reported range for human elastin, 6–13 residues/1,000). The amino acid analyses of collagen, elastin, supernatant, and pellet residue were performed using ion exchange chromatography (Model 7300 Amino Acid Analyzer, Beckman) for desmosine and isodesmosine determinations and by high-pressure liquid chromatography after phenyl-thio carbamyl (PTC) conversion for hydroxyproline determinations.

**Fluorescence Microscopy of Normal and Atherosclerotic Aorta**

Fluorescence microscopy was used to visually evaluate fluorescence localization in normal and atherosclerotic aorta. Frozen cross sections of cadaveric normal and atherosclerotic human aorta (10 μm thick) were illuminated using a 325-nm helium-cadmium laser and imaged through a microscope (model BH-2, Olympus). Photomicrographs were taken using an OM-2 camera body (Olympus) and ET135 film (Kodak) (exposure time, 4–16 minutes) for subsequent analysis of fluorescence localization. These sections were then formalin fixed, and after histologic processing, were stained with Richardson’s combination of Verhoff’s
Figure 2. Laser-induced fluorescence emission spectra of collagen, human elastin, cholesterol, heparan sulfate, hyaluronic acid, and chondroitin sulfate. Spectra are normalized to peak intensity for comparison. The spectrum of bovine elastin (not shown) was identical to that of human elastin.

elastic and Gomori’s trichrome stains and evaluated by light microscopy for correlation with the fluorescence photomicrographs.

Spectral Classification Algorithm

A classification algorithm was developed to discriminate normal and atherosclerotic aortic spectra based on an elastin and collagen spectral decomposition. Laser-induced fluorescence spectra had previously been obtained from 91 normal and 91 atherosclerotic human cadaveric aortic specimens. Normal aortic specimens had an intimal thickness of less than 200 μm. Atherosclerotic aortic specimens had a plaque thickness greater than 900 μm and consisted of fibrous or mixed fibrous and fatty lesions. Calcification and cholesterol clefts were frequently present. The spectra from these specimens were evaluated by the spectral classification algorithm developed in this study. Fifty normal and 50 atherosclerotic spectra were grouped into a training set, and the remaining 41 normal and 41 atherosclerotic spectra were grouped into a validation set. Each aortic spectrum was normalized to unit area and decomposed into a linear combination of pure elastin and collagen spectra (normalized to unit area) using a least-squares error criterion (Aortic spectrum=E×elastin spectrum+C×collagen spectrum). Spectral coefficients for elastin and collagen (E and C, respectively) were constrained to values ranging from 0 to 1. A discriminant score was formed by the difference of elastin and collagen coefficients (E−C) to classify aortic fluorescence spectra. Initially the E−C spectral classification algorithm was applied to the training set of 50 normal and 50 atherosclerotic aortic spectra. The threshold E−C score was determined by the score that classified the training set with the greatest accuracy. The spectral classification algorithm was then prospectively applied to the validation set of 41 normal and 41 atherosclerotic aortic spectra to assess the prospective classification accuracy of this algorithm.

Statistical Analysis

All results, unless otherwise stated, are expressed as mean±1 SEM. An unpaired Student’s t test was used to compare the E−C scores of the normal and atherosclerotic aortic spectra.

Results

Fluorescence of Known Constituents

The fluorescence emission spectra of collagen, elastin, cholesterol, and glycosaminoglycans are shown in
Figure 2. Calcium phosphate and calcium hydroxyapatite did not have recorded fluorescence; the fluorescence emission could not be reliably differentiated from background noise. The individual fluorescence spectra are shown normalized to peak intensity.

Visual inspection revealed a similarity between the fluorescence of elastin and that of normal artery as well as a similarity between the fluorescence of collagen and that of atherosclerotic artery. Collagen and elastin spectra are shown respectively with representative atherosclerotic and normal aortic spectra (from Reference 14) in Figure 3.

**Lipid, Collagen, and Elastin Extraction**

Representative laser-induced fluorescence spectra from specimens of normal aorta and aortic atherosclerotic plaque subjected to homogenization and lipid, collagen, and elastin extraction are shown in Figure 4. Homogenization of the tissue did not change the tissue fluorescence spectrum. Removal
of lipid also did not substantially alter the fluorescence spectra of either normal aorta or atherosclerotic plaque, suggesting a small contribution of lipid fluorescence to net arterial fluorescence. The spectrum of lipid fluorescence, when present, was similar to the cholesterol fluorescence spectrum shown in Figure 2. The fluorescence spectra of the pellet residue (extracted elastin) of normal and atherosclerotic specimens were similar, and they closely approximated the recorded spectrum of commercially purified elastin. There were no demonstrable differences in the fluorescence of the elastin residue from normal aorta and from atherosclerotic plaque. The fluorescence spectra of the supernatant (extracted collagen) of normal and atherosclerotic tissue were similar to that of commercially purified collagen, except that the spectrum of the supernatant from normal aorta was slightly broader.

Amino acid analysis confirmed that the pellet residues were composed of elastin. The average isodesmosine and desmosine content was 17 residues/1,000 for the pellet from normal aorta and from atherosclerotic plaque. Amino acid analysis confirmed that the supernatant of autoclaved plaque was 91% collagen. The supernatant of autoclaved normal aorta was 67% collagen, probably because of the presence of other solubilized proteins or of possible contamination by elastin. This latter possibility was evidenced by the presence of a small amount of desmosine in the supernatant specimens of normal aorta. This could account for the broader fluorescence spectra of the supernatant from autoclaved normal aorta compared with that of pure collagen.

Fluorescence Microscopy

The most intensely fluorescent structures in normal aorta and in the aortic media of atherosclerotic aorta were the elastic fibers. The most intensely fluorescent structures in the atherosclerotic plaque were the collagen fibers, which were especially abundant in the fibrous cap. In addition, there were occasional, deeper fluorescence structures in the atherosclerotic plaque that corresponded to lipid deposits on light microscopy. Figure 5 shows light and fluorescence photomicrographs of representative sections of atherosclerotic human aorta.

Spectral Classification Algorithm

The spectra from the 50 normal and 50 atherosclerotic aortic specimens that made up the training set were decomposed into a linear combination of elastin and collagen spectra. The mean coefficients were E=0.91±0.02 and C=0.08±0.02 for normal aorta and E=0.25±0.03 and C=0.72±0.02 for atherosclerotic aorta. The linear combination of elastin and collagen spectra closely approximated the actual arterial fluorescence spectra; the root mean square error of the estimated intensity was 53 intensity units for atherosclerotic spectra and was 31 intensity units for normal spectra (average peak intensity, 765 and 478 units, respectively). The mean E–C value for normal aorta was therefore +0.83±0.04, and for atherosclerotic aorta, it was −0.48±0.07. These mean values were significantly different at a p value less than 0.001. Analysis of the training set data revealed that an E–C threshold cutoff value of 0.3 optimally classified (e.g., greatest accuracy) the training set. Aortic spectra with an E–C score greater than 0.3 were classified as normal, whereas those with an E–C score equal to or less than 0.3 were classified as atherosclerotic. Classification accuracy with this criterion was 92% for the normal specimens and was 90% for the atherosclerotic specimens. Prospective application of the E–C spectral classification algorithm to the 82 spectra in the validation set revealed a classification accuracy of 98% for normal aortic spectra and 93% for atherosclerotic aortic spectra. This resulted in an overall prospective classification accuracy of 93%. The distribution of E–C scores is shown in Figure 6.

Discussion

With the current investigations into the potential of laser angioplasty,20,21 there has been renewed interest in the fluorescence of atherosclerotic and normal arterial tissue. Laser-induced fluorescence spectroscopy appears to be a promising imaging modality to differentiate atherosclerotic from normal artery and therefore could be incorporated in a “smart” laser angioplasty system to enable selective targeting of laser energy to plaque and thus avoid vessel perforation. An understanding of the causes of the difference between normal and atherosclerotic arterial fluorescence will enhance the application of fluorescence guidance. Using 325-nm excitation, this study identified elastin and collagen as the major arterial fluorophores of intact normal and atherosclerotic human aorta, respectively. This conclusion is based on the analysis of the 325-nm induced fluorescence spectra of known constituents of normal and atherosclerotic arteries, fluorescence analysis of lipid, collagen, and elastin extracts from normal and atherosclerotic arteries, and analysis of arterial fluorescence photomicrographs. Consistent with this explanation for the causes of arterial fluorescence, a classification algorithm based on elastin and collagen spectral decomposition was developed that accurately classified normal and atherosclerotic arteries.

Earlier studies isolated fluorescent compounds in atherosclerotic plaque. Blankenhorn et al10,11 identified four types of fluorescence associated with plaque and attributed two types of green fluorescence to carotenoids, which are pigmented lipids known to accumulate in atherosclerotic plaque. Prince et al8 also extracted a yellow substance from atherosclerotic plaque that had spectral properties consistent with carotenoids. This substance had a green fluorescence. Banga7,8 isolated a fluorescent elastin-related substance from atherosclerotic aorta that he termed “atherofluorescent compound.” He
FIGURE 5. Representative light (Panel A) and fluorescence (Panel B) photomicrographs of atherosclerotic aorta. The most intensely fluorescent structures in the fluorescence photomicrograph correspond to elastic fibers in the media and to collagen fibers in the atherosclerotic plaque in the light photomicrograph of the same section stained with Richardson's stain.²⁰

postulated that degeneration and damage of elastin during atherosclerosis led to the formation of this fluorophore. None of these studies, however, correlated these fluorophores with the observable fluorescent emissions from intact normal or atherosclerotic arteries.

The present study shows that elastin is the major fluorophore in normal aorta and that collagen is the major fluorophore in atherosclerotic aorta. The fluorescence spectra of the extracted elastin from normal and atherosclerotic artery did not differ. The fluorescence spectra of the extracted collagen from

FIGURE 6. Elastin-collagen spectral classification algorithm. A histogram distribution of the E-C scores for the 91 normal (hatched bars) and 91 atherosclerotic (shaded bars) aortic spectra (training and validation sets) are shown. The dotted line represents the threshold cutoff value of 0.3. Classification accuracy of all 182 spectra was 92%.
normal and atherosclerotic aorta were also similar. This indicates that the fluorescence difference between normal and atherosclerotic artery is related not to a difference in collagen or elastin structure, but to a difference in the relative concentrations of collagen and elastin. This hypothesis is consistent with the reported collagen and elastin composition of normal and atherosclerotic artery. Rokosova et al. documented a 14-fold difference in the collagen-to-elastin ratio of normal artery compared with atherosclerotic plaque (collagen-to-elastin ratio, 0.5 for normal artery and 7.3 for plaque). Noble similarly reported that the collagen content of early atheromas was 143% that of normal, and the collagen content of advanced atheroma was 172% that of normal. Smith confirmed these results in fibrous plaque in which noncalcified lesions had 164% and calcified lesions had 254% of the collagen content of normal artery.

Fluorescence spectroscopy of known constituents of normal and atherosclerotic artery revealed that despite the potentially high calcium content of atherosclerotic plaque, calcium compounds (phosphate and hydroxyapatite) do not fluoresce and thus do not contribute to atherosclerotic arterial tissue fluorescence. Although chondroitin sulfate, heparin sulfate, and hyaluronic acid had fluorescence emission, these glycosaminoglycans account for less than 2% of the organic matrix of normal artery and compose 0.4% of the organic matrix of atherosclerotic plaque. Therefore, these compounds probably would not substantially contribute to the fluorescence of intact arterial specimens unless their fluorescence quantum yield was much greater than that of other constituents. The fluorescence of the lipid extracted from normal aorta and atherosclerotic plaque was similar to the fluorescence of cholesterol. Despite the reported moderate lipid content of atherosclerotic plaque and normal artery (18.4% and 6.9% of the organic matrix, respectively), lipid appeared not to contribute substantially to atherosclerotic or normal arterial fluorescence.

The possibility does exist that additional fluorophores contribute to intact arterial fluorescence in other types of atherosclerotic plaque. Specifically, carotenoids that have been localized from fatty plaques fluoresce maximally at 535 nm and may contribute to yellow fatty plaque fluorescence. This study was limited to the analysis of fluorescence from thick fibrous or fibrofatty plaques. A broader range of atherosclerotic subtypes (e.g., ulcerated or necrotic atheroma) may reveal other major plaque fluorophores. The present study also does not take into account fluorescence selfabsorption by plaque chromophores. The phenomena by which emitted tissue fluorescence is reabsorbed by other tissue compounds will probably have a substantial influence on the fluorescence of hemorrhagic atherosclerotic plaques (e.g., because of hemoglobin absorption characteristics).

The elastin-collagen spectral classification algorithm correctly classified aortic arterial tissue specimens with a high degree of accuracy. In general, algorithms based on arterial composition are preferred to algorithms based on empiric pattern recognition. With approaches such as this, it may be possible to obtain information about arterial composition from the intact arterial spectrum. For example, the E and C spectral coefficients may be correlated with actual elastin and collagen content. This would require knowledge of the fluorescence characteristics and yield of these substances in intact arteries and the possible effect of self-absorption.

There are several other limitations of this study. The extraction experiment and classification algorithm were applied to aortic tissue and may reveal different results when applied to arterial tissue from other sites. This would be particularly relevant when comparing the aorta, which is an elastic artery, with coronary arteries that are muscular arteries having a lower elastin-to-collagen content ratio. Although the elastin-to-collagen content ratio of normal coronary artery also exceeds that of atherosclerotic plaque, it remains to be confirmed that collagen and elastin are also the major fluorophores of atherosclerotic and normal coronary artery, respectively. This study correlated the fluorescence of laboratory reagents with that of intact in vitro cadaveric tissue. Not only may in vivo arterial fluorescence differ, but the fluorescence of arterial constituents may be affected in vivo by the local environment (e.g., pH and protein conformation). The difference between fluorescence of compounds in powder reagent form and in intact arteries is suggested by observations on collagen and elastin fluorescence. In powder form, collagen had a fluorescence intensity two and a half times greater than elastin (L. Laifer, personal observation). However, Leon et al. have reported greater fluorescence intensity in “elastin rich” normal artery than in “collagen rich” atherosclerotic plaque.

The interpretation of arterial fluorophores in this study is based on an excitation wavelength of 325 nm. Thus, collagen and elastin are major arterial fluorophores using a fluorescence excitation wavelength in the ultraviolet range. Other fluorophores may predominate when excitation sources at other wavelengths are used to induce arterial fluorescence. However, Rava et al. reported that structural proteins had a substantial contribution toward arterial fluorescence with an excitation wavelength of 476 nm, but their study did not separate the individual contributions of elastin and collagen fluorescence.

In conclusion, collagen and elastin are the major fluorophores of aortic atherosclerotic plaque and normal artery, respectively, and the difference between normal and atherosclerotic arterial fluorescence appears to be due to differences in relative collagen and elastin content. Consistent with this observation, a classification algorithm based on elastin and collagen spectral decomposition can
accurately classify normal and atherosclerotic aortic fluorescence spectra.

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References

19. Richardson L: Richardson’s combination of Verhoff’s elastic and Gomori’s trichrome stains with modifications. Lab Med 1975;6:33–34

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