

## Abstract

It is reported that immunostaining of myoglobin (Mb) is useful for forensic diagnosis. In this study, we investigated the condition of fixation of striated muscle in 10% neutral-buffered formalin to obtain appropriate stationarity of Mb in immunostaining. Firstly, criteria for staining intensity and definition of the stainability of examined were determined for sheep muscle tissue. Sheep myocardial tissue was fixed using 10% neutral-buffered formalin under the 21 different conditions based on combinations of the following: three ratios of volume of formalin (mL) to weight of myocardium (g) (RFM) of 1, 4 or 9, 7 durations of fixation (DF) of 0.5, 3 or 6 hours, and 1, 2, 5 or 7 days. Secondly, detection of myoglobin diffused from skeletal muscle from autopsy cases into formalin during fixation were confirmed by ELISA. Finally, the evaluation of stainability of Mb of striated muscle in routine autopsy examinations was confirmed using sheep staining intensity standards. From this experimental investigation, it has been demonstrated that the most suitable formalin fixation condition for using myoglobin staining in forensic diagnosis is RFM4 with a fixation time of at least DF 3 hours up to 1 day. It was evident that staining intensity decreases with fixation durations exceeding 2 days, irrespective of the RFM. Thus, the fixation time was deemed the most influential factor affecting the staining properties of myoglobin staining in skeletal muscle tissue. When conducting myoglobin staining using striated muscle as an evaluation sample, particular attention should be paid to the fixation time.