Step Sectioning Protocol for Comprehensive Evaluation of Cardiac Valves When Using a Mouse Model

M. T. O'Brien[1], E. M. Edmonds[1], L. Kuhn[1], R.K. Jacoby[1]
[1]Charles River Laboratories, Frederick, Maryland, USA

ABSTRACT

Introduction: Cardiac valves are essential for normal transcardiac blood flow and are vulnerable to test article-related changes. However, routine histologic sectioning for standard toxicologic cardiac evaluation in mice may not necessarily include sufficient cardiac valve tissue to detect valvular changes if present. Thus, in studies having a high index of suspicion for possible drug-induced valvulopathy (e.g. class effect), multiple sections of heart can be evaluated to ensure that all valves are adequately examined for test article-related changes.

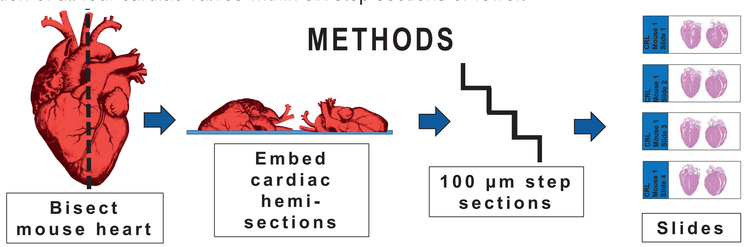
Experimental Design and Methods: Twelve (12) C57/Bl6 formalin-fixed mouse hearts were submitted for histologic evaluation. Three (3) mice belonged to the control group; the remaining nine (9) mice were divided into three different groups (n=3 mice/group) receiving the test article of a class known to induce valvulopathy. Serial step sectioning was performed on bisected paraffin-embedded hearts at 100 µm intervals. The presence of valve leaflet tissue and valve identity were evaluated to determine how many step sections were necessary for comprehensive cardiac valvular evaluation.

Results: In 10 of 12 mice (83%), portions from all cardiac valve leaflets (right atrioventricular valve, RAV; left atrioventricular valve, LAV; aortic valve, AoV; pulmonic valve, PV) could be observed within six step sections or fewer.

Conclusion: Bisecting the heart with step sectioning at 100 µm intervals enabled examination of all four cardiac valves within six step sections. Subjectively, slightly smaller step intervals (50-75 µm) may also be useful when evaluating mouse cardiac valves.

Impact Statement: Step sectioning of bisected mouse hearts at 100 µm intervals generally provides for examination of all four cardiac valves within six step sections or fewer.

METHODS



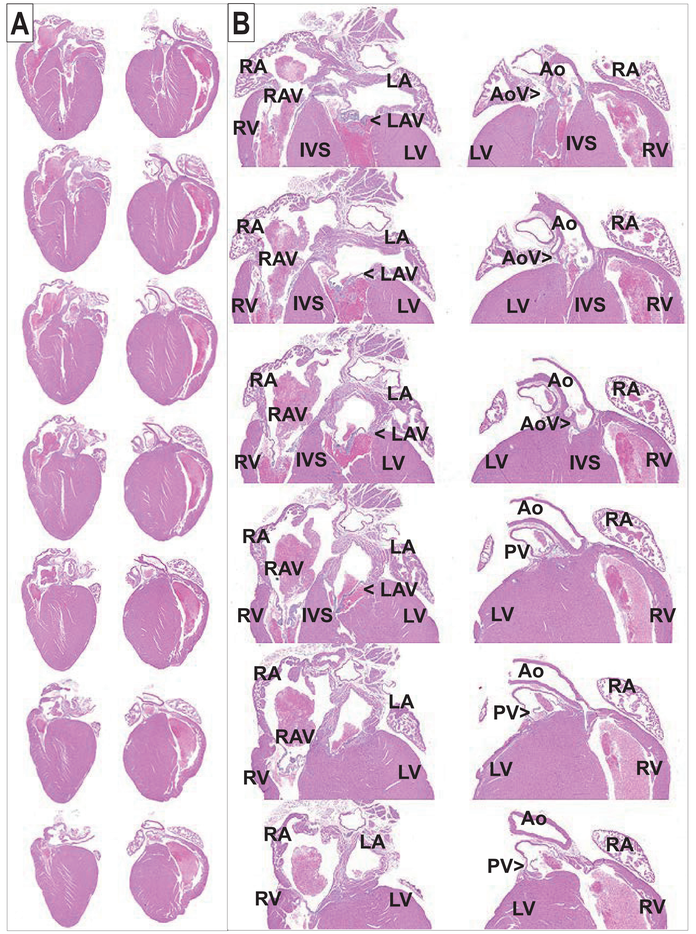
CONCLUSIONS

- Step sectioning of bisected mouse hearts at 100 µm intervals generally enabled serial assessment of all cardiac valves (RAV, LAV, AoV, PV)
- Serial sectioning of cardiac valvular tissue is useful for identifying subtle valvular trends (i.e. whether valve morphology changes are sectioning variability or authentic thickening)
- Serial sectioning of cardiac valvular tissue may not be necessary if valve changes are dramatic
- Smaller step intervals (50-75 µm) may also be useful for capturing all valves

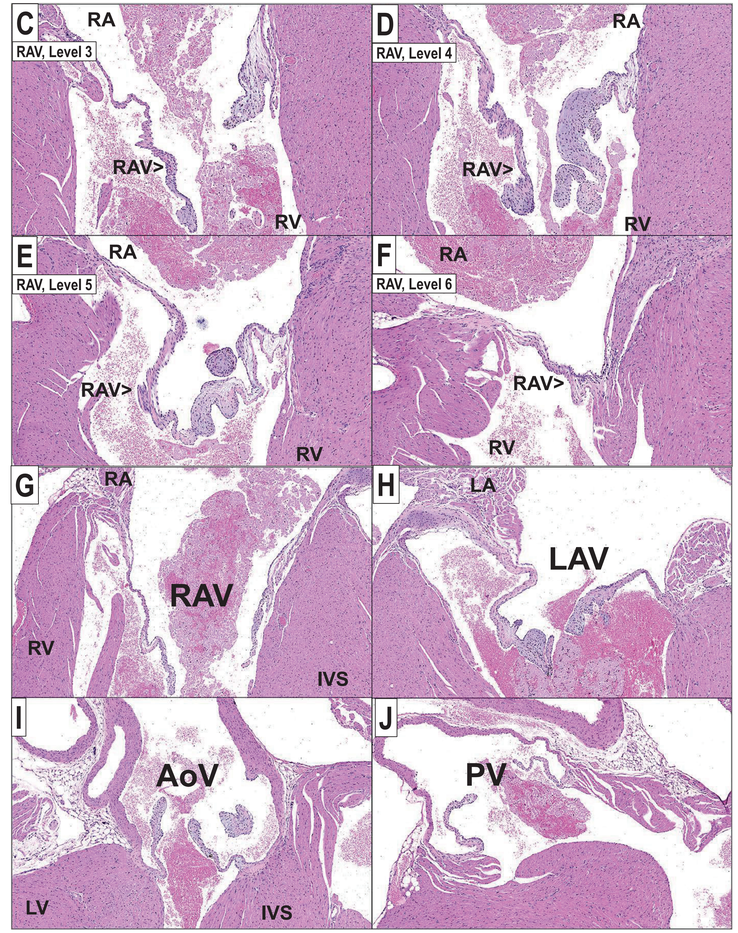
RESOURCES

Anderton MJ, Mellor HR, Bell A, et al. **Induction of heart valve lesions by small-molecule ALK5 inhibitors**. Toxicol Pathol 2011;39:916-924.

HISTOLOGY AND DISCUSSION



Figures A&B: Bisected mouse heart, serial cardiac step sections at 100 µm, H&E; **A.** Subgross, whole heart, 1x**, B.** Higher magnification of cardiac valves, with relevant cardiac anatomy indicated, 2x. RV/LV: right/left ventricle; RA/LA, right/left atrium; RAV/LAV, right/left atrioventricular valve; AoV, aortic valve; PV, pulmonic valve, IVS, interventricular septum



Figures C-F (H&E, 8x): C-F: Serial step sections of RAV highlighting variability of valvular appearance between 100 µm interludes; H&E, 8x.
Figures G-J (H&E, 7x): Higher magnification of individual cardiac valves: **G.** Right atrioventricular valve, RAV; **H.** Left atrioventricular valve, LAV, **I.** Aortic valve, AoV, **J.** Pulmonic valve, PV