Intramuscular Injections in Rabbits: Improving Tissue Collection and Assessing Multiple Tissue Sections for Accurate Histopathologic Evaluation

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OBJECTIVES

To improve the accuracy of injection site histopathology through enhanced tissue collection methods and multiple section assessment.

INTRODUCTION

Intramuscular (IM) injections sites are commonly used for vaccine studies because of the abundant blood supply and resultant rapid and uniform absorption of test compounds.[1] Rabbits have historically been used as the common model for these safety studies because they mount a robust immune response to vaccine administration and are large enough to test a full human dose.[1] For histopathological examination of IM dose sites, the sections should include all major tissues associated with the selected dose site (skin, subcutaneous tissue, and muscle).[2] However, delivering complete and consistent histopathologic sections of IM injection sites in rabbits within a high production laboratory environment can be challenging. For many test articles, the primary histopathological findings may be limited to the immediate injection site region, and factors such as loose attachment of rabbit skin to the underlying muscle and acquisition of only single sections contribute to unacceptable margins of error for injection site identification, collection, and evaluation.

Lepage et al., (2012) reported that the quality of diagnosis for IM injection studies was not enhanced by examination of more than one section. However, that study did not employ a specific injection site identifier and used degeneration and/or inflammation for site identification. Using a persistent adjuvant allows differentiation from nonspecific findings that may be seen away from the injection site and may not be directly associated with test article administration. Thus, accurate assessment of the actual injection site is of high importance.

A series of test IM injections using aluminum phosphate adjuvant (Alum) allowed assessment of the successful recovery and examination rate of IM lumbar musculature injection sites in rabbits under various conditions. The tested parameters included improvements in collection technique, anatomical orientation, and microscopic examination of multiple sections.

RESULTS

Table 1: Summary of Alum-positive injection sites in IM-injected rabbits, with comparison between single and multiple tissue section examination, before enhanced tissue collection methods update

	Single Section	Multiple sections		
		Total Animals		
Number of Animals	**60**	**60**	**42**	**18**
Total # Sections Evaluated/ Animal	**1**	**7 or 12 (Combined)**	**7**	**12**
Injection Site 1	18 **(28%)**	39 **(65%)***	23 **(55%)***	16 **(89%)***
Injection Site 2	8 **(22%)**	47 **(78%)***	30 **(71%)***	17 **(94%)***
Injection Site 3	6 **(10%)**	24 **(40%)***	15 **(36%)***	9 **(50%)***
Injection Site 4	2 **(3%)**	10 **(17%)***	5 **(12%)***	5 **(28%)***
Combined Injection Sites	**34/240 (14%)**	**120/240 (50%)***	**73/168 (43%)***	**47/72 (65%)***

*** Statistically significant increase in incidence ($p < 0.05$) compared to single-section examination**

No enhanced tissue collection or dosing procedure was used.

Table 2: Summary of Alum-positive injection sites in IM-injected rabbits, comparing individual and multiple section examination, following enhanced tissue collection methods update

	Total Animals with Alum identified (n=20)	Average Percentage of Sections Affected	Most Common Individual Section Affected+ (Percent affected)
Total # Sections Evaluated/ Animal	**5**	**5**	**5**
Injection Site 1	17 **(85%)***	**53%**	**D (70%)**
Injection Site 2	20 **(100%)***	**61%**	**D (85%)**
Injection Site 3	18 **(90%)***	**45%**	**C (65%)**
Injection Site 4	17 **(85%)***	**56%**	**A (65%)**
Combined Injection Sites	**72/80 (90%)***	**54%**	**71%**

*** Statistically significant ($p < 0.05$) increase in incidence compared to individual section examination**

\+ Sections were sampled from cranial to caudal, labeled A to E, respectively. Section C was located at the central portion of the marked skin, with other sections spaced at 5–10mm intervals.

METHODS

Two studies were performed to evaluate the accuracy and consistency of injection site identification, collection, and assessment in rabbit intramuscular injections. All microscopic assessment of injection sites used lumbar epaxial injection sites (4/animal) injected with Alum adjuvant, producing characteristic microscopic findings (Fig 1). While almost every animal had microscopic findings (inflammation, etc.) at the injection sites, only those with Alum present (Fig. 1) were listed as positive. A student's T-test was performed to assess statistical significance ($p<0.05$).

Sixty New Zealand white rabbits were IM-injected biweekly with 0.6 mg of Alum in saline (0.5 mL dose). The skin at the dose site was marked with indelible marker. No specialized methods of site selection and injection or collection of the marked injection site were used.

- A single section was taken through the center of the skin-marked injection site for initial microscopic assessment.
- Multiple additional sections from the original single-section tissue block and fixed tissues surrounding the original section (5–10 additional sections) were examined secondarily.

Twenty New Zealand white rabbits were IM-injected biweekly with 0.125 mg of Alum in saline (0.5 mL dose). The skin at the dose site was marked with indelible marker. Enhanced dosing and tissue collection procedures, including specific anatomical localization of dose sites, tissue immobilization before fixation, and multiple section sampling were used as described in the text box to the right.

- Five sections from each marked injection site (taken from cranial to caudal) were surveyed, and findings in each section tallied separately and in combination.

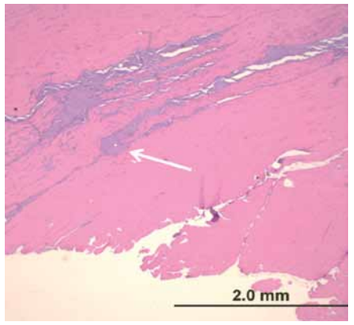

Figure 1: Intramuscular Alum injection site—IM injection site with characteristic Alum adjuvant finding of basophilic macrophages (white arrow) dissecting between muscle fibers, but can be seen in the deep subcutis as well. H&E stain at 20X.

Enhanced tissue collection of common IM injection sites (Figures 2–4)

A separate project to improve tissue handling and fixation, and improve dose site accuracy and consistency, was undertaken as part of the MPI Research internal technician certification program, and new procedures were set in place based on the resulting data.[3]

Briefly, New Zealand white rabbits were used to develop specific anatomical landmarks and animal holding procedures, and assess shifting of the skin at the time of dosing, tissue collection, and fixation.

- IM injection of dye (post-euthanasia) using palpable anatomical markers (e.g., ribs, spine, and pelvis) was used to improve methods of dosing, postmortem site localization, and collection.
- Shifting of skin position relative to underlying muscle was dramatic upon fixation, but was ameliorated by immobilization of the tissues at the time of collection (Fig. 2 and 3).
- IM dye location was assessed macroscopically following fixation (Fig. 4).

Figure 4: Ink Injection used for test injection site identification—Successfully identified IM injection delivery in the central three sections trimmed through the marked skin surface (arrows indicate injected black dye).

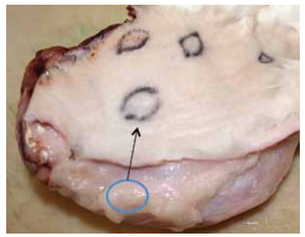

Figure 2: Formalin fixation-associated skin contraction at hind limb—Skin contracts asymmetrically from muscle. The actual IM injection site (blue circle) is 3–4 cm (arrow) from the associated skin dose site.

Figure 3: Excised dose site—The cut edge of the skin was secured to the cut edge of the muscle with a surgical stapler. Injection sites thereby fixed with minimal skin movement.

CONCLUSIONS

- Sampling multiple sections of the injection site produces statistically significant 3–4 fold increases in positive site identification without the use of enhanced tissue collection methods.
- Using enhanced tissue collection methods produces notable increases in positive site identification (almost double).
- There is variance in which individual section has the most findings, and not every section has findings; therefore, examination of 5 or more sections is recommended.
- Combining enhanced anatomical specificity in dosing; systematic, specialized injection site collection and tissue handling; and microscopic evaluation of multiple tissue sections provides accurate injection site identification rates of 85–100% at common IM injection sites, whereas single section evaluation is significantly less accurate (25–85%).

REFERENCES

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