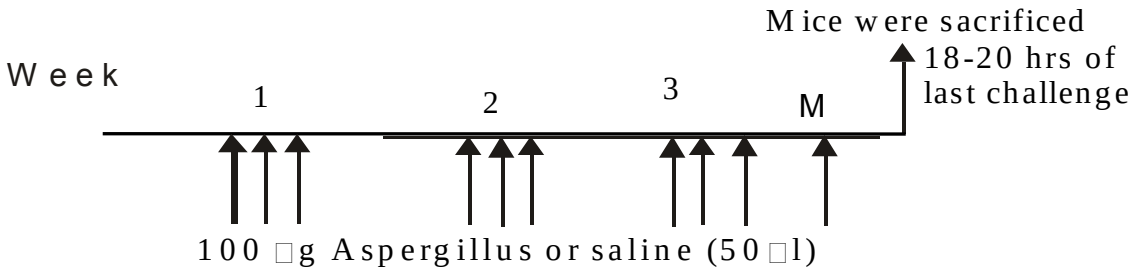
Modified Protocol.

A mouse model of allergic EE is established using methods described previously with few modifications [1]. In brief, mice are lightly anesthetized with isoflurane (Iso-Flo; Abbott Laboratories, North Chicago, IL), and 100 μg of allergen protein (50 μl saline+0.05% glycerol) of *Aspergillus fumigatus* (Lyophilized, GREER) or 50 μl of normal saline+0.05% glycerol alone is applied to the nares using a micropipette with the mouse held in the supine position. After three treatments per week of total 3.5 weeks with one additional challenge, mice are sacrificed between 18 and 20 hours after the last intranasal allergen or saline challenge.



Please note that the source of *Aspergillus fumigatus* is critical in inducing esophageal eosinophilic inflammation. Our experience indicates that allergenic properties and protein concentration of the allergen vary from source and even batch to batch. We recommend first, to determine the protein concentration of the allergen extract and second, to perform a pilot study to test the Aspergillus preparation. Notably, when we established this model we were using the clinical Aspergillus from Bayer Corporation, Spokne, Washigton. The protein concentration during that time was ~100 mg/ml. This Aspergillus extract is no longer available. Recently, we tested a number of Aspergillus extracts from different sources and found each to have allergenic activity but at variable levels. We now

recommend *Aspergillus fumigatus* extract from GREER, Laboratories, Lenoir, NC for experimental EE studies.

Additional important steps to be considered.

1. Mice should be unconscious during the procedure. The mouse tongue should have no muscle activity; otherwise, the allergen will be swallowed and will not promote allergic responses.
2. Mice should not gasp during challenges.
3. Mice should be in a supine position during the challenges, avoid any folding of the neck.

Example photomicrograph of experimental EE