Title: Cat Pelt/Hair Extract US Potency: Updating the Predicate Fel d 1 RID with a mAb-based ELISA

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Rationale: Cat pelt/dander and hair extracts used for skin testing and immunotherapy are compared to the US potency standard based on their Fel d 1 content using a polyclonal goat antibody-based radial immunodiffusion (RID) assay. As an alternative, we have developed a quantitative two-site ELISA that uses high-affinity Fel d 1-specific monoclonal antibodies (mAbs).

Methods: Fel d 1-specific mAbs were produced by IgGenix starting from plate-based single-cell RNA sequencing of IgE producing B cells isolated from cat-allergic individuals. DNA encoding Fel d 1-specific CDRs were cloned into human IgG4 cassettes and mgs of 4 anti-Fel d 1 mAbs were produced, 2 reactive to each of 2 discrete Fel d 1 epitopes. All 4 mAbs were biotinylated and analyzed by box titration as purified/biotinylated cross-pairs to assess the pair producing optimal performance.

Results The mAb pair that produced the widest ELISA working range and lowest limit of detection was purified clone IGX-0201 (capture) and biotinylated-clone IGX-0204 (detection). Extensive cross-validation using 10 masked commercial cat pelt/hair extracts (range 4 to 20 U/ml, Fel d 1) in two laboratories by multiple testers produced inter-RID/ELISA method coefficients of variation CVs from 3-19% with intra- and inter-ELISA assay %CVs of <10% and <15%, respectively. Regression correlation demonstrated excellent agreement of Fel d 1 concentrations as measured by the predicate RID and new ELISA (***Y=0.88X+0.63, r =0.95***).

Conclusion. The tight correlation between Fel d 1 levels determined by RID and a new mAb-based ELISA supports the adoption of the new ELISA for use in future commercial cat extracts.