A. **Scope-**The purpose of the following test was to determine if the product, EasyDECON™ (EFT DF 200), was capable of neutralizing the antigenic portion of two specific molds, *Aspergillus fumigatus* and *Alternaria alternata*, for the *Asp f1* and *Alt a1* antigens.
B. **Referenced Documents**-There were no specific standards that we are aware of for this type of analysis.
C. **Terminology-**
   a. Antigen-A chemical or compound that elicits an allergic response in a susceptible individual. A chemical or compound that reacts with a monoclonal or polyclonal antibody specifically developed for the detection of the chemical or compound.
   b. q.s.-Abbreviation for Quantum Sufficit. The process of adding a solute or reagent to achieve a final desired volume.
D. **Significance and Use-** The fungi were grown under controlled conditions, the spores harvested, and then treated with the product following the manufacturer s instructions. The subsequent materials were then tested to determine if the antigens, *Asp f1* and *Alt a1* were present in the sample. Control samples were run in parallel with the test samples to insure that the antigenic properties of the organisms were not affected by any other procedures during the test.
   If the test product works according to the manufacturer s expectations, then there should be no detectable allergens in the test solutions for either of the fungi.
   The samples must be adequately neutralized after exposure to the product to stop all activity by the product and prior to the allergen analysis to avoid interference by the product with the allergen analysis. The allergen data are reported in micrograms of allergen per milliliter of spore solution. The spore solution was quantified and data are reported in Colony Forming Units per milliliter of spore solution.

E. **Reagents and Materials-**

   a. **Spore growth medium-**Malt Extract Agar (MEA) with 0.1% chloramphenicol, Health Link Catalog #1085
   b. **Phosphate Buffered Saline (PBS) pH 7.4-**10X solution, Fisher Chemicals catalog # BP399-1
   c. **Alternaria alternata (TX 8025)-**Remel catalog # 4601016
   d. **Aspergillus fumigatus (KM 8001)-**Remel catalog # 4601018
   e. **Lazy-L-Spreaders-**Hardy catalog # SPR-L-S10
   f. **50 ml sterile, non-pyrogenic, polypropylene centrifuge tube with plug seal caps-**Biologix catalog # BCT-P15RS-370
   g. **15 ml sterile, non-pyrogenic, polypropylene centrifuge tube with plug seal caps-**Biologix catalog # BCT-P50RS-370
   h. **Alt a1 ELISA kit (121/121)-**Indoor biotechnologies
   i. **Asp f1 ELISA kit-**Indoor biotechnologies

F. **Procedures-**

a. Spore Collection:
   i. Alternaria alternata (TX 8025)-Remel catalog #4601016 and Aspergillus fumigatus (KM 8001)-Remel catalog # 4601018 were inoculated on MEA.
   ii. Well grown (after 7-10 days, incubated at 23°±3°C) Alternaria alternata and Aspergillus fumigatus culture plates were chosen for harvesting the spores.
   iii. 5 ml of sterile PBS (Lot # 032904-PBS-D1) was added to the each culture plate
   iv. The cultures were harvested using sterile Lazy-L-spreaders (Lot # 3301 from Hardy) by gently rubbing the top of the colony to dislodge the spores.
   v. The spore mixture was collected in sterile 50 ml Falcon tubes.
   vi. Each solution was q.s. to 35 ml using sterile PBS.
   vii. The spore solution was vortexed to break the spore clumps.
   viii. 1 ml of spore solution was transferred into 0.9ml dilution tubes with 0.1 % Tween (Lot # 032404-10T-A) for plating. The samples were serially diluted from 10^{-7} to 10^{-15} per ml.
   ix. The dilutions were plated onto MEA plates (Lot# 0405852) to determine the amount of spores present in the solution.

G. The spore solution was vortexed and 5 ml of the solution was transferred into 15 ml centrifuge tubes.
   a. Six aliquots each were prepared for Alternaria alternata and Aspergillus fumigatus. Three aliquots of each organism were used for the controls and three aliquots of each were used for the test.

H. The samples were centrifuged for 5 minutes and the supernatant was discarded.

I. Preparation of EasyDECON™ test solution. The solution was prepared following manufacturers instructions.
   a. For 100ml of EasyDECON™ used in the test.
      i. 49 grams of Penetrator was added to a tared 200 ml sterile beaker.
      ii. 49 grams of Fortifier was added to a tared 200 ml sterile beaker.
      iii. 2 grams of Booster was added to a tared 200 ml sterile beaker.
      iv. Added 49 grams of Penetrator to 49 grams of Fortifier and mixed thoroughly for a minute and the added 2 gram of booster for a final weight of 100 grams.

J. Testing Procedure

a. 1 ml of EasyDECON™ was added to three Alternaria alternata and three Aspergillus fumigatus tubes.
b. 1 ml of sterile water was added to three Alternaria alternata and three Aspergillus fumigatus tubes to act as the control samples.

c. The samples were incubated for 1 hour at room temperature.
d. After the incubation period the samples were centrifuged and the supernatant was discarded.
e. The samples were washed with 2 ml of sterile water and centrifuged. After the centrifuge process the supernatant was discarded.
f. The washing and centrifugation was repeated twice to remove any leftover product.

K. **Testing for Allergens:**

a. 300 µl of extraction buffer was added to each tube of the *Aspergillus* tubes and 1 ml of extraction buffer was added to each of the *Alternaria* tubes. The samples were incubated for 2 hours on a rotating mixer (LabQuake from Barnstead/Thermolyne)
b. The samples were centrifuged for five minutes, the supernatants were collected, and the pellets were discarded.

L. The standard EMLab protocol was used to perform the ELISA analysis.

M. **Quality Assurance/Quality Control**- Controls for each sample were run in triplicate. The controls followed the entire process with the exception of the exposure to the product. All temperatures and incubation times were recorded in a laboratory notebook, along with all other specific information pertaining to the testing procedure.

N. Results

The following spores concentration were used for the tests:

Alternaria alternata-5ml x (3.2×10^8 CFU/ml)=1.6×10^9 spores per test

Aspergillus fumigatus-5ml x (2.2×10^9 CFU/ml)=1.1×10^{10} spores per test

The following average concentrations of allergens were found:

Test Alt a 1 = < 0.8 µg/ml

Test Asp f 1= <0.8 µg/ml

Control Alt a 1 = 32.15 µg/ml

Control Asp f 1 = 19.23 µg/ml

0.8 µg/ml is the detection limit for these allergen analyses, and so therefore no Alt a 1 and Asp f 1 allergens were detected in the test samples using the EMLab ELISA methodology.