Chapter 18. SPECIES IDENTIFICATION FIELD TESTS (SIFT)

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18.1 Introduction

A series of individual, serological screen tests has been developed for rapid species verification of raw whole/ground meat tissue or emulsified meat products in field environments. They are collectively referred to as the Species Identification Field Tests (SIFT). The individual tests which comprise SIFT are as follows: ORBIT (Overnight Rapid Bovine Identification Test), PROFIT (Poultry Rapid Overnight Field Identification Test), PRIME (Porcine Rapid Identification Method), SOFT (Serological Ovine Field Test), REST (Rapid Equine Serological Test), and DRIFT (Deer Rapid Identification Field Test).

The basis of these tests is that of an agar-gel immunodiffusion technique using stabilized reference antigen and antibody reagent impregnated paper discs and prepared agar-gel plates that have a printed template for correct placement of test components. Identification of a species tissue is demonstrated by a reaction of complete fusion between sample and reference antigen immunoprecipitin bands which become plainly visible after overnight incubation of the immunodiffusion plate at room temperature. Key components are stable for at least one year when stored under refrigerator conditions. Each test has been shown to have adequate sensitivity and specificity for its intended purpose of the particular species in question. These tests are reliable, practical, economical, and very easy to perform and interpret in any work environment. Individual species tests for beef, pork, poultry and sheep are commercially available as a complete test kit. As a result of an Association of Official Analytical Chemists (AOAC) collaborative study, the method of these tests is an official AOAC first action method.

18.2 Materials and Methods

All materials necessary for the performance of SIFT for beef, pork, poultry and sheep species may be commercially purchased as individual test kits. The method of performing SIFT for beef species detection using an ORBIT test kit is described below. Performance of SIFT for other species, using the other SIFT kits available, would be conducted in an identical manner except for the substitution of the appropriate dye colored - template marked agar-

gel plates and species reference antigen and antibody reagent discs relative to the species being tested. Specific formulations for preparation of the agar-gel plates and the reference antigen and antibody reagent discs for each species SIFT kit are detailed in the individual references cited at the end of this protocol.

18.21 ORBIT Kit Composition is as Follows:

- **a. ORBIT agar-filled plates with pink dye; pattern for disc placement silk screened on plate bottom.**
- **b. Vial of Anti-Beef Antibody Discs-A-.**
- **c. Vial of Beef Reference Antigen Discs-B-.**
- **d. Vial of Blank Discs-S-.**
- **e. One piece flat black construction paper.**
- **f. Three pieces of white paper.**
- **g. One felt-tip marking pen.**
- **h. Polyethylene sample bags.**
- **i. Three forceps.**
- **j. Hyperion viewer (optional accessory).**

18.22 Ground Meat Accessory Kit Composition is as Follows:

- **a. Wooden applicator sticks - six inches long.**
- **b. Sample cups - silk screen printed with two permanent measurement lines on outside.**
- **c. Forceps.**

18.3 Procedure

- **a. Remove prepared ORBIT agar-gel immunodiffusion plates and reagent discs from the refrigerator and allow equilibration to room temperature.**

- **b. Using the forceps carefully place one anti-beef antibody disc, flat on the agar surface, such that the A lettered circle of the template is completely and evenly covered by the disc.**

- **c. In an identical manner place one beef reference antigen disc over the B lettered circle of the same plate.**

- **d. Sample discs may be prepared from either thawed whole muscle tissue or from ground/formulated meat products:**

i. If the sample is whole tissue, make a vertical slice about 38 mm deep in an area which is free of fat or connective tissue. With clean forceps place one blank sample disc halfway into the depth of the slit and gently squeeze the slit closed such that both sides of the disc are in contact with the tissue. Let the disc remain in this position 10 - 30 seconds to absorb tissue fluids and appear obviously wet.

ii. If the sample is of a ground/formulated type, place about 1 gram well packed into the sample cup such that it is filled level with the bottom black measuring line. Add sufficient quantity of cold tap water to fill the beaker level to the top black measuring line. Mix sample and water with a clean wooden applicator stick such that a uniform emulsion results. Tilt the cup 45° and with clean forceps immerse a blank sample disc in the emulsion to a depth necessary for complete saturation. Excess fluid and meat particles are removed from the disc by wiping it on a cup rim during removal.

e. The sample disc, from either type of sample is placed over one of the S lettered circles of the ORBIT plate containing the reference discs.

f. Treat a second sample in an identical fashion and place that disc over the remaining unoccupied S lettered circle of the same plate.

g. Tightly seal the lid on the plate and leave undisturbed overnight (15 - 24 h) at room temperature.

h. The plates are then examined with an indirect white light source against a flat black background. This may be done with a Hyperion viewer or by using black paper taped to and suspended vertically from the rear part of a desk lamp's housing.

i. Examine the plate for the formation of characteristic immunoprecipitin lines in the agar among the four discs to determine which sample contain beef.

18.4 Results

Immunodiffusion reactions for the ORBIT test are interpreted as are those for other SIFT plate reactions. A reference band should always be visible between the reference antigen-B- and reference antibody-A- discs. Complete fusion of this line with a band formed between the antibody-A-disc and the sample-S-discs is indicative of a positive reaction for that sample. Absence of a band between the sample and the antibody disc is read as negative. Any lines formed near the sample disc that are not extensions of the reference band are also negative reactions.

18.5 Quality Control Procedures

a. Maintain storage of unused prepared plates and reagent discs at refrigeration conditions (4°C) in order to assure adequate shelf life and proper reactivity. DO NOT FREEZE.

b. Do not use any kit components beyond their expiration date.

c. Use separate, clean forceps for each individual disc placement to prevent reagent or tissue fluid carry over and cross contamination.

d. Proper disc placement and positioning is critical to obtaining expected reactions.

e. An immunoprecipitin band must always be produced between the reference antigen and antibody discs, as this serves as the positive control and assures the proper reactivity of the test system. If a reference band is not produced, the test system is invalid, samples should not be interpreted and the cause of the failure to produce the reference band must be determined and corrected before subsequent testing can proceed.

f. Do not attempt to read any immunodiffusion plates that have reacted for more than 24 h.

g. The normal room temperature for proper incubation of immunodiffusion plates is considered to be in the range of 70 - 78°F (21.1 - 25.6°C).

18.6 Selected References

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