PRELIMINARY REPORT ON SOLUBLE ANTIGEN FLUORESCENT ANTIBODY TECHNIQUE FOR IDENTIFICATION OF HOST SOURCE OF MOSQUITO BLOOD MEALS[1]

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The ability to identify the host source of blood meals in captured engorged mosquitoes is a valuable aid in studies involving mosquito-borne diseases transmitted to man and other vertebrates. The precipitin test is normally used for obtaining this information but has remained largely unsatisfactory due to the time and effort expended, the difficulty often encountered with weak reactions and false positives, and because of the requirement for training and experience in interpretation of results. Due to these factors there is a desire among many workers for a simpler, more accurate method for determining mosquito blood meals.

Recent advances in fluorescent antibody techniques (Toussaint and Anderson, 1965) permitting use of soluble antigens, significantly extended the capabilities and versatility of immunofluorescence procedures. The basic methodology developed for this procedure, and used in a direct rather than indirect immunofluorescence technique, appeared to offer promise as a

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simple, precise and sensitive method for identifying the host source of mosquito blood meals. With this method, tests can be easily read either visually under UV light of appropriate wavelength or mechanically with a fluorometer. In addition, the technique requires only small amounts of material, thereby increasing the number of tests that can be performed on a given sample. Furthermore, the simplicity of the procedure facilitates rapid testing of large numbers of specimens and the adaptability of the technique to field conditions. These factors, plus the present availability of commercially prepared fluorescein-conjugated antisera against many animal species, prompted the investigations described herein.

METHODS AND MATERIALS

PREPARATION OF REAGENTS. TenX stock Tris buffer (pH 8.0) was prepared by dissolving 121.14 grams of Tris (hydroxymethyl) aminomethane in one liter of distilled water. The pH was adjusted to 8.0 with about 600 ml N/1 hydrochloric acid and sufficient distilled water to make two liters was added. TenX stock NaCl (8.5 percent) was prepared by dissolving 170 grams NaCl in two liters of distilled water. Buffered saline of 0.05 M Tris (TrBS) was prepared by mixing 200 ml. of 10X stock Tris buffer and 200 ml. of 10X stock NaCl solution and adding sufficient distilled water to make two liters.

Conjugated antisera to be tested were diluted (usually 1:5 to 1:20) with TrBS containing 2 percent Tween 80 (Polyoxyethylene (20) Sorbitan mono-oleate).

PREPARATION OF ANTIGEN. Blood for testing was obtained by allowing laboratory reared female *Anopheles quadrimaculatus* and *Aedes aegypti* to feed on various animals. Stomach contents were smeared on filter paper immediately after the mosquitos became engorged and at intervals up to 24 hours. After the blood smears were allowed to air dry at ambient temperature they were stored in a household type refrigerator for periods up to 2 months prior to testing.

The soluble antigen was prepared by cutting small round pieces of filter paper containing the dried blood smear, placing them into depressions of a Disposotray and adding 0.2 ml. of TrBS directly on top of the blood smear. The smear was agitated with an applicator stick at the beginning and end of a 10-minute period. An alternate method of elution was to use a mechanical shaker which eliminated manual agitation and reduced the elution time to 5 minutes.

MATRIX PREPARATION. The matrix for the soluble antigen was prepared by punching ¼-inch diameter discs from cellulose acetate filter paper (Millipore Type HA, white, grid surface, pore size 0.45 microns) with a hand-operated paper punch. The discs were separated from the protective paper and numbered on the grid side with India ink, care being used throughout to prevent contact between fingers and the filter paper.

TEST PROCEDURES. Cellulose acetate discs were held at the edge with forceps and dipped plain side down into the saline extracted antigen for a few seconds until wet, removed and placed plain side up on clean filter paper. Control discs were prepared by dipping in TrBS without antigen. A desk lamp with a 60 watt bulb was placed 6–8 inches above the discs for a drying period of 15 minutes. Properly diluted conjugated antisera were placed in clean depressions in 0.2 ml. amounts and the dried discs were placed plain side down in the conjugate and allowed to incubate at room temperature for 30 minutes. Conjugated antisera were then removed with an aspirator or pipette and the discs washed immediately in 1 ml. TrBS for 5 minutes. This was followed immediately by two more TrBS washes, one of 10 and one of 5 minutes, making a total of 20 minutes washing time. The discs were removed, dried for 15 minutes, and placed plain side up on the sticky surface of masking tape or black plastic electrical tape, and placed in a dark box or room under appropriate UV light (Portable short wave, "Mineralite," 2537 Å). Results were read by first determin-

ing that control discs were negative, as indicated by a uniform dark blue to purple color. Test discs were checked for fluorescence that varied from bright yellow-green (strong reaction) to a dull brown-green (weak reaction). Negative discs appeared as negative controls.

RESULTS

Tests have been conducted with *Anopheles quadrimaculatus* and *Aedes aegypti* to identify blood meals from chickens, humans and monkeys up to 24 hours after feeding. During tests of more than 100 blood smears only two samples have given negative results; one monkey blood taken 16 hours post feeding and one chicken blood taken 24 hours after feeding, the latter being stored more than 4 months prior to testing. As shown in the table there is some cross-reaction between human and monkey antigens but homologous series have so far produced strong reactions when compared to heterologous series which have given weak reactions. These preliminary results indicate that in areas where cross-reactions occur, identifications can be made easily and accurately if paired antisera are used.

Attempts to identify blood taken from mosquitoes more than 24 hours after feeding have not been made, nor have tests been conducted to identify material collected in the field.

Trials have shown that from 15 to 20 cellulose acetate discs can be coated with antisera from a single sample allowing a wide spectrum of antisera to be tested.

The experiences of Toussaint and Anderson (1965) and those of Fife and Toussaint (personal communications), plus a single test we have conducted, indicate good possibilities for quantitative measurement of antigen-antibody reactions even when results are not detectable visually. Future experiments are planned for quantitative measurements, especially in areas where cross reactions occur.

Identification of blood meals from ten mosquitoes collected at varying intervals following feeding (tested as unknowns)

ANTIGENS	ANTISERA		
	human	monkey	chicken
Chicken (0)[1]	—[2]	—	++
Chicken (8)	—	—	++
Chicken (16)	—	—	++
Chicken (24)	—	—	—
Human (0)	+	—	—
Human (8)	+	—	—
Human (24)	+	—	—
Monkey (0)	±	++	—
Monkey (0)	+	++	

[1] Hours after blood meal.
[2] — = negative, ± doubtful, + weak positive, ++ positive.

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Literature Cited

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