APPENDIX IX

Hematology Procedure

Field Operations Manual

NIOSH Contract No. 210-76-0175

I. Hemoglobin Determination.

Quantitative estimation of hemoglobin is used as a routine test to detect the existence and/or degree of anemia. In the hemoglobin determinations red blood cells are lysed to release the hemoglobin fraction. The hemoglobin is then quantitatively converted to cyanmethemoglobin by the addition of Drabkin's Reagent.

Reagents.

A. Drabkin's Reagent

Sodium bicarbonate	1.0 gm
Potassium ferricyanide	0.2 gm
Potassium cyanide	0.005 gm

Dissolve in distilled water and dilute to one liter. Store in a dark bottle. The solution is stable for one month if protected from light and evaporation.

Caution: Cyanide salts and solutions are poisonous and should be handled carefully. Pipette solutions with a bulb. Mix solutions by swirling. If any of the compounds are spilled, clean them up quickly and carefully. When disposing of solutions in the sink, wash down generously with cold water.

Method for Cyanmethemoglobin Determination.

A. Calibration of Fisher hemoglobin detector.

Use the commercial standard (Hycel). The undiluted standard in this method represents 20 gm. percent of hemoglobin.

1. Prepare cuvettes as follows:

Volume of Standard	Volume of Drabkin's Reagent	Gm % Hgb
6 ml	2 ml	15
2 ml	6 ml	5

2. Set the Hi control knob on the machine at 15 with the 15 gm % standard and the Lo knob at 5 with the 5 gm % standard.

3. Read the Fisher artificial standards (in sealed tubes) against the hemoglobin standards.

4. Plainly label the Fisher standard tubes with the values thus obtained.

B. Hemoglobin Method.

1. Dispense 5 ml of Drabkin's Reagent in Fisher cuvettes.

2. Add 0.02 ml of whole blood to the solution (1-25- ml dilution).

3. Set the Hi and Lo knobs at the values indicated on the sealed artificial standards provided with the instrument.

4. Insert the cuvette with test sample and determine the hemoglobin in gm % on the direct reading scale.

II. Oxyhemoglobin Determination.

In this measurement hemoglobin is converted to oxyhemoglobin in the presence of dilute or weak alkali solutions. This determination measures active hemoglobin; hence, the values may be lower than cyanmethemoglobin in the same samples.

Reagents.

A. 0.04% Ammonium Hydroxide

Dilute 0.4 ml of concentrated NH_4OH to 1.0 liter using distilled water

Method for oxyhemoglobin determination.

1. Calibrate the detector as described previously.

2. Dispense 5.0 ml of 0.04% NH_4OH into cuvettes.

3. Add 0.02 ml of capillary or venous blood.

4. Mix well.

5. Read immediately or within 24 hours.

III. Hematocrit Determinations.

Method

1. Select capillary tubes approximately 7 cm long with a 1.0 mm internal diameter.

2. Using capillary action fill the tube with blood to within 1.0 cm of the end.

3. Plug one end with plasticine.

4. Centrifuge the tubes in a microhematocrit centrifuge at 5000 xg for 10 minutes.

5. Measure the length of the blood column, including the plasma.

Normal values.

Hemoglobin	Hematocrit
Adult females 12-16 gm %	42 ± 5
Adult males 14-18 gm %	47 ± 7

APPENDIX X

Antigen Preparation

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Rationale:

The presentation of a foreign substance to the immune system elicits the production of antibodies which are directed toward the foreign substance. The nature of the antibody produced, in part, determines the immunopathology of the disease. The production of antibodies of the IgE class would result in immediate allergic reactions upon subsequent challenge. Conversely, the production of antibodies of the IgG class and, occasionally of the IgM class, would favor the elicitation of a hypersensitivity pneumonitis reaction in the lung on re-exposure to the foreign substance.

It is possible to ascertain the presence of antigen specific IgE antibodies which evoke allergic reactions by intradermal skin testing (Appendix XI) and the presence of IgM or IgG antibodies directed toward specific antigens by precipitation (Appendix XII). The determination of both the allergic antibody and the precipitating antibodies depend heavily on the preparation of extracts from organic material. Extracts from organic material can be prepared from pulverized washed grains, grain dusts, pulverized grain insects and from culture filtrates of bacteria and fungi known to cause hypersensitivity pneumonitis.

Reagents:

1. The panel of organic material used for saline extraction of antigenic materials:

Intact Grains	Respirable Grain Dust	Insects and Mites
Durum wheat	Durum wheat	Adult granary and rice weevils-mix
Spring wheat	Spring wheat	
Barley	Barley	
Corn	Corn	Confused flour, Dermestid and Black Carpet Beetle-mix
Rye	Rye	
Oats	Oats	
Sunflower seeds	Sunflower Seeds	Mold, house and grain mite-mix
Small seeds		
Soybeans		
Culture Filtrates Thermophilic bacteria		Culture Filtrates Fungi
Micropolyspora faeni-UW		A. fumigatus-1
Micropolyspora faeni-Marshfield		A. fumigatus-5
Micropolyspora faeni-Greer		A. fumigatus-6
		A. fumigatus-9
T. candidus-Medical College of Wisconsin		A. fumigatus-1022
		A. flavus
T. candidus-UW		A. niger

Culture Filtrates Thermophilic Bacteria	Culture Filtrates Fungi
T. sacchari	A. clavatus
	Aureobasidium
T. vulgarus-Marshfield	Alternaria species
T. vulgarus-Hollister/ Steir	c. albicans
	Cephalosporium
T. viridans	Fusarium
Settled dust	Hormodendrum
	Mucor
	Phoma
Settled dust I-Rafter Dust	Trichoderma
Settled dust II- Holding tank	P. casei
Settled dust III-Dump Station	P. rubrum
Hay and Dusts	Other antigen
Moldy Hay	Pigeon serum
House Dust	

B 0.85% Saline
C Whatman No. 1 filter paper
D Dialysis tubing
E Coca's non-allergenic buffer (Hollister-Steir Laboratory)
F Glycerine
G Sterile dropper vials - 2.0 ml (Greer Laboratories)
H 0.1 M borate citrate buffer pH 8.4 (Appendix XII).

Methods:

1. Preparation of saline extracts of grain, respirable grain dust, insects and mites.

   a. It is necessary to prepare a fine powder of intact grains and insects. Intact grains can be pulverized in a Ball mill, whereas insects and mites can be ground into a fine powder with a mortar and pestle. The growth conditions for insects and mites are shown in Table I.

   b. The powder is suspended in 0.85% saline in a 1:10 W/V ratio and incubated with constant agitation at 4°C for 24 hours.

   c. The mixture is allowed to settle for 2 hours at 4°C and the supernatant fluid removed.

   d. The supernatant fluid is filtered through a Buchner filter using Whatman No. 1 filter paper.

e. The effluent is placed in dialysis tubing and dialyzed against running, cold tap water for 24 hours.

f. After dialysis, the extract is concentrated to 20-50 ml. in the dialysis tubing by perevaporation.

g. The concentrated extract is placed in a new dialysis tubing and dialyzed against 0.85% saline at 4°C for 24 hours.

h. The extract is lyophilized by conventional methods.

i. The protein content of each extract is determined by Micro-Kjeldahl protein determinations (A10.1). Results are expressed as protein nitrogen units per mg. of lyophilized material.

j. Store lyophilized extracts in a -20°C freezer.

2. Preparation of saline extracts from culture filtrates of bacteria and fungi.

a. The broth cultures of each organism are grown as shown in Table II.

b. The broth culture is centrifuged at 1000 xg for 30 minutes at 4°C.

c. The supernatant fluid is carefully decanted into a beaker without disturbing the precipitate.

d. The supernatant fluid is filtered through Whatman No. 1 filter paper using a Buchner funnel.

e. The effluent is placed in dialysis tubing and dialyzed against cold, running tap water for 24 hours.

f. The remaining steps of the procedure are identical to those described for preparation of grain, respirable grain dusts and insects and mites.

3. Preparation of immediate skin test reagents.

a. Remove extracts of grain, grain dusts, and insects from the freezer.

b. Using sterile technique, prepare 50 ml of sterile Coca's buffer containing 50% glycerine v/v.

c. Using the PNU/mg determinations, weigh out 200,00 PNU of each lyophilized extract.

d. Add each extract to separate sterile dropper bottles. Label each bottle with the name of the extract and the date.

e. Using sterile technique, add 2.0 ml of the Coca's buffer with 50% glycerine. Note that the fluid concentration is 100,000 PNU/ml.

f. Solubilize the extracts by gentle agitation.

g. Store the reconstituted extracts at 4°C.

4. Preparation of extracts for determination of precipitating antibodies.

   a. Remove lyophilized extracts of grain, respirable grain dust, insects and the culture filtrates of bacteria and fungi from the freezer.

   b. Weigh out 15 mg of each of the extracts.

   c. Place extract in a small 2.0 ml screw-topped vial. Label the vial with the name of each extract and the date.

   d. Add 1.0 ml of the 0.1 ml borate citrate buffer pH 8.4 to each vial and replace the screw top. Note that the final concentration of antigen is 15 mg/ml.

   e. Store the reconstituted extracts in a refrigerator.

Normal or Reference Values:

The reference values, in terms of protein nitrogen units (PNU) for grain, respirable grain dusts, insects and settled dusts are shown below.

	Intact Grains		Respirable Dust	
	PNU/ MG	µg Protein/ µg of Solid	PNU/ mg	µg Protein/ µg of Solid
Durum Wheat	12000	760	5600	350
Spring Wheat	12600	790	5800	325
Barley	12600	790	4300	270
Corn	8200	515	3600	225
Rye	8000	500	5000	310
Oats	11300	710	5000	310
Sunflowers	12700	790	2900	180
Small Seeds	7000	435	---	---
	Insects			
Weevils-Mix	12900	810		
Beetles-Mix	9900	620		
	Settled Dust			
Settled Dust I	---	---	5300	300
Settled Dust II	---	---	4600	290
Settled Dust III	---	---	5100	32

Limitations of the Procedure:

Most organic antigens can be extracted into saline and this method has proven suitable for extraction of antigenic material from culture filtrates of bacteria and fungi known to cause hypersensitivity pneumonitis. One must be aware, however, that the metabolic antigens produced by bacteria and fungi differ quantitatively and qualitatively during growth. Therefore, careful consideration must be given to the dynamics of antigen production by each organism and the cultures must be harvested during peak antigen production. The quantitation of antigens can be achieved by two dimensional cross-immunoelectrophoresis using a human serum with known serological reactivity to the metabolic antigens (A10.2). Samples of culture fluids can be assayed at weekly intervals and the cultures harvested when there is an increase in the length of precipitin arcs or in the numbers of precipitin arcs. Since there is some batch to batch variation in serological reactivity of culture filtrates from bacteria and fungi, it is necessary to produce enough of each extract to test on control populations with the same batch of antigen extract.

There is also variation in serological reactivity within strains of the same species of bacteria or fungi and/or the immune response to these organisms is strain specific. These phenomena have been observed in precipitin analyses using extracts from A. fumigatus, Penicillium and the thermophillic actinomycetes. It is, therefore, necessary to include in the screening panel for precipitating antibodies several extracts of different strains of the same species of certain bacteria and fungi.

Extracts of the thermophillic bacteria and certain fungi were not included in the panel used to ascertain immediate skin test reactivity. Many of these extracts, particularly the thermophillic bacteria, evoke toxic skin reactions which render them useless in determining skin reactions. Moreover, these agents usually evoke hypersensitivity pneumonitis which lacks a true allergic immunological component. Hence, even if the extracts were suitable for use in immediate skin tests, one would expect negative skin tests with these extracts.

The use of saline extracts of grain and respirable grain dusts for immediate skin tests has a major limitation. Many of the proteins of grain are insoluble in saline, including the entire gluten complex consisting of both gliadin and glutenin. Approximately 80% of the endosperm protein is associated with the gluten complex (A10.3). The endosperm constitutes 70% of the total protein in the grain. Only the albumin and globulin fraction are extracted with saline. Hence, the saline extracts of grain used for skin tests measures the presence of atopy to albumin or globulins and not the atopic potential of other grain proteins.

REFERENCES
Appendix 10

A10.1 Kabat, EA and Mayer, MM: Experimental Immunochemistry, C.C. Thomas Co., Springfield, IL, 1961, p. 476.

A10.2 Weeke, B: Crossed Immunoelectrophoresis, Scand. J. of Immunol., 1973, 2(Suppl 1):47.

A10.3 Nerxheimer, H: The hypersensitivity to flour of bakers apprentices. Acta. Allergol. (KbH), 1973, 28:42.

TABLE I. The Growth Conditions for Insects and Mites.

		Growth Media
Granary Weevils Adults	Sitophilus granarius	Whole Wheat
Rice Weevils Adults	Sitophilus Oryzae	Whole Wheat
Confused Flour Beetle Adults and Larvae	Tribolium confusion	Whole Wheat with 5% Brewers Yeast
Black Carpet Beetle Larvae	Attagenus megatoma	Purina Lab Chow with 5% Brewers yeast
Dermestid Beetle	Trogoderma glabrum	8 Purina Lab Chow* 3 Wheat Germ 3 Dry Milk 1 Brewers yeast 1 Meat & Bone Meal
Mold Mite	Tyrophagus putrescentiae	3 Brewers Yeast* 1 Wheat Germ
House Mite	Glyophagus domesticus	3 Brewers Yeast 1 Wheat Germ
Grain Mite	Acarus species	3 Brewers Yeast 1 Wheat Germ

*weight:weight ratio

All insects were maintained at 27 ± 1°C and 60% relative humidity with a 16:8 Light:Darkness photo ratio. All insects were screened from the growth medium and then examined microscopically for remaining media. When necessary, the insects were rescreened and further separated from remaining media. The insects were then promptly frozen and stored in a -40°C freezer.

TABLE II. Growth Conditions for Bacteria and Fungi.

	No. 1 liter Prescription Bottles	Growth Media	Amount of Media (ml)	Incubation Temp	Incubation Time Weeks
Asp. fumigatus 1*	48				
Asp. fumigatus 5*	48				
Asp. fumigatus 6*	48	Czapek-Dox	200	37°C	3
Asp. fumigatus 9*	48				
Asp. fumigatus 1022**	48				
Asp. flavus	48				
Asp. niger	48				
Asp. clavatus	48	Czapek-Dox	200	Room temp	4
Aerobasidium	48	Czapek-Dox	200	Room temp	5
Alternaria	48	Czapek-Dox	200	Room temp	5
C. albicans	48	Sabourauds Broth	200	37°C	5
Cephalosporium	48	Sabourauds Broth	200	Room temp	5
Fusarium	48	Czapek-Dox	200	Room temp	5
Hormodendrum	48	Czapek-Dox	200	Room temp	5
Mucor	48	Czapek-Dox	200	Room temp	5
Phoma	48				
Trichoderma	48	Czapek-Dox w/ 30 g/L dextrose	200	Room temp	5
Moldy Hay		Saline extract			
House Dust		Saline extract			
M. faeni-Marshifield					

TABLE II (continued)

M. faeni-U.W.		Trypticase Soy Broth***		56°C	1
M. faeni-Greer Labs					
T. sacchari		Trypticase Soy Broth***		56°	1
T. candidus-Med. Coll. Wis.		Trypticase Soy Broth***		56°	1
T. candidus-U.W.		Trypticase Soy Broth***		56°	1
T. iridans		Trypticase Soy Broth***		56°	1
T. vulgaris-Marshfield		Trypticase Soy Broth***		56°	1
T. vulgaris-Hollister Steir		Trypticase Soy Broth***		56°	1
T. vulgaris-Marshfield		Trypticase Soy Broth***		56°	1
Pen casei	48	Czapek-Dox	200	Room temp	5
Pen. rubrum	48	Czapek-Dox	200	Room temp	5
Pigeon Serum		---		---	-

*Isolated from sputum cultures

**American type culture collection

***Double dialysis technique of Edwards (Med. Lab. Technol., 28:172, 1971)

APPENDIX XI

Skin Testing Protocol

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RATIONALE

It is possible to reproduce both allergic and cell mediated immune reactions by introducing small amounts of antigenic material into the skin. Based on the time course of the reaction, the skin tests can be classified as immediate reactions which occur within 20 minutes and delayed reactions which are noted after 24-48 hours. Although both skin test reactions have similar morphologies, the immunological mechanisms which mediate the reactions are different. The immediate skin test reaction is due to the interaction of allergens with antibodies of the IgE class which are bound to mast cells and basophils in the skin. The allergen antibody complex intiates the release of histamine, SRS-A and other mediators from the mast cells and basophils. The pharmacologically active agents increase vasoconstriction and increase vascular permeability which results in a localized area of edema called a wheal which is surrounded by a less defined area of redness called an erythema. Since allergen specific IgE is only found in allergic individuals, the immediate skin test is a direct measure of the allergic or atopic status of the test subject. Moreover, in respiratory allergies, skin tests are often positive when respiratory challenge fails to reproduce the disease (A11.1).

The prick test is the method of choice for determining immediate skin test reactivity in large population studies. The method is reasonably safe with minimal systemic antigen absorption. Moreover, one can perform large numbers of tests without discomfort to the test subject. Unlike the intradermal tests, nonspecific reactions seldom occur with the prick test.

The delayed skin reaction is the result of the interaction between sensitized lymphocytes and antigen introduced into the skin. The sensitized

lymphocytes are, in effect, memory cells which remember previous exposure to selected bacterial, viral and fungal agents (i.e. tuberculosis, mumps, streptococcal infection, Candida and trichophyton infections). Upon introduction of these antigens into the skin, sensitized lymphocytes localize in the skin test area and release small molecular migration and metabolic activity of other cell types including the peripheral blood monocytes. Monocytes with increased metabolic activity are localized in the skin test area and initiate an acute inflammatory response. The inflammatory response results in localized swelling called induration surrounded by an area of erythema. Hence, the delayed skin test reaction to selected antigens is a measure of the functional status of the cell mediated immune system. It follows that the delayed skin reactivity reaction is useful in determining whether there is decreased delayed hypersensitivity if reactions are observed it would suggest that exposure to environmental agents caused an immuno-suppresive effect.

Principle:

The immediate skin tests measure the allergic sensitivity to common areo-allergens and possible occupation-related environmental allergens. Conversely, the delayed skin tests measure the status of the cell mediated immune system as measured by the capacity to mount an inflammatory response to microbial, viral and fungal antigens.

Reagents:

I. Immediate Skin Tests

a. Prick test reagents for immediate skin tests (Appendix VI)

Common* Allergens	Fungal* Extracts	Grain Insects
Giant/Small Ragweed	Aspergillus fumigatus	Adult grain & rice weevils - mix
Timothy Grass	Penicillium species-mix	
Mixed feathers	Aspergillus species mix	Adult confused flour, blank carpet and dermestid beetle - mix
Eastern Oak	Mucor	
Cat epithelium	Cladosporium werneckii	
Oak Rust	Alternaria herbarum	Mold, house & grain mite - mix
Grain smut		
Insect Grains	Airborne Grain Dust	Settled dust
Durum wheat	Durum wheat	Settled Dust I
Spring wheat	Spring wheat	Settled Dust II
Barley	Barley	Settled Dust III
Corn	Corn	
Rye	Rye	
Oats	Oats	
Sunflower seeds	Sunflower seeds	
Small seeds		
Soybeans		
Positive Control	Negative Control	
1.0% histamine in diluting fluid with 50% glycerine	Diluting fluid with 50% glycerine	

b. Sterile stainless steel needles

c. Alcohol impregnated pads

d. 2x2 gauze pads

e. Magic markers

f. Drug box with resuscitation equipment and adrenalin (1:1000 V/V)

*Purchased from Greer Laboratories, Lenoir, NC

II. Delayed Skin tests

a. Skin test panel for delayed skin testing

PPD - tine test

SK/SD - 4U/IU in - 0.1 ml

Mumps - 0.1 ml of stock

Candida - 10 PNU in 0.1 ml

Trichophyton - 1:1000 dilution in -0.1 ml using Δ 1:10 w/v stock solution

b. Tuberculin syringes with 27 gauge needles

c. Alcohol impregnated pads

d. 2x2 gauze pads

e. Corticosteroid impregnated tape

Methods:

A. Immediate skin tests

1. Subject removes shirt or blouse and lies face down in a horizontal position.
2. The back is cleansed with alcohol impregnated pads.
3. Using a magic marker, the numbers 1-10 are painted in two columns approximately 3" from and on either side of the spine. The numbers should begin near the shoulder and terminate near the waist. By placing extracts on either side of both columns, it is possible to test 40 different extracts.
4. The skin test reagents are placed in rack rows of 10. The initial sample in the first row should be the positive histamine control and the last sample in the test panel should be the negative control.
5. Using the column of numbers nearest the technician, a single drop of extract numbered 1-10 is placed on the back.

6. Using the opposite side of the same numerical column, a drop of extracts 11-20 are placed on the back, beginning at the shoulder and working downward.
7. With a sterile needle the skin is gently scratched beneath each drop. The needle is directed so that the skin is slightly raised as the needle punctures the skin. Clean the needle with a 2x2 gauze pad between tests.
8. Wipe the back clean of extracts 1-20 using 2x2 gauze pads.
9. Using the column of numbers farthest from the technician, place drops of extracts 21-30 on the back.
10. Using the opposite side of the same column of numbers, place a drop of extracts 31-40 on the back.
11. Repeat step 7.
12. Repeat step 8.
13. The subject is given a laboratory timer and asked to report back to the technician after 20 minutes.
14. The largest axis of the wheal and erthyema is determined using a ruler graduated in millimeters.

B. Delayed skin tests

1. Sterile 1.0 ml tuberculin syringes are loaded with 0.15 ml of each antigen. Bubbles are removed from the barrel by gentle agitation. The plunger is then pressed until only 0.1 ml remains in the syringe barrel and the needle.
2. Ask the test subject to roll up sleeves to the elbow.
3. Cleanse the forearms with alcohol impregnated pads and allow to dry.
4. Using the left forearm, inject intradermally 0.1 ml of three compounds (Candida, mumps, PPD) in alphabetical order beginning near the elbow joint.
5. Using the right forearm, inject 0.1 ml of the remaining two compounds (SK/SD and trichophyton) in alphabetical order, beginning near the elbow.

6. The test subject is asked to return in 48 hours.
7. The longest axis of the induration and erythema is determined using a ruler graduated in millimeters.

Normal or Reference Values:

In the course of the study, a positive immediate skin test reaction was considered to be a wheal ≥3.0 MM and/or erythema greater than 5.0 MM. Positive control histamine induced skin reactions were greater than 5.0 MM wheal.

Because of the variability in the potency of allergenic extracts, it is impossible to give reference value for the frequency of positive immediate skin tests in a population. Hence, for scientific validity the frequency of immediate skin tests in a test population should be compared to the frequency of skin tests in a similar-sized control population.

When the delayed skin tests were used in the study the induration and erythema was measured after 48 hours. The criteria for positive delayed skin reactions were:

Antigen	Induration	Erythema
Candida	≥ 5.0 MM	≥ 15.0 MM
PPD	≥ 10.0 MM	
SK/SD	≥ 5.0 MM	
Trichophyton	≥ 5.0 MM	

The frequency of delayed skin reactions to intermediate strength antigens used in the study has been defined in the general population (A11.2).

Antigen	%
Candida	39
Mumps	78
PPD	26
SK/SD	55
Trichophyton	28

Limitations:

The use of the prick test for skin testing has one disadvantage. The prick test is less sensitive than intracutaneous skin testing. Hence, allergy cannot be ruled out on the basis of a negative prick test (A11.3).

Conversely, persons with some skin conditions will respond to all skin test reagents. These individuals usually present with skin dermography of several types. These individuals can be identified by virtue of the fact that they will also have a positive response to the diluting fluid. Although the individuals should be excluded from the data pool, it is sometimes possible to demonstrate true allergic reactions which are greater than reactions observed with the diluting fluid (A11.3).

It is also possible that some individuals will not respond to the positive histamine control. This may be due to certain medications ingested by the subject or poor technique (A11.3). If the subject has taken medications which influence histamine action, he should be excluded from the data pool.

The delayed skin tests also have several theoretical and practical limitations. First, it is conceivable that the test population has not been exposed to the test antigen(s). Hence, one would observe a decreased frequency of positive reactions within the population. It is necessary, therefore, to determine the frequency of positive delayed skin test reactions in control population of similar size from the same geographic area. Second, certain immunopathological processes preclude demonstration of a positive delayed skin test. Hence, persons with atopic dermatitis should be excluded from the study. Third, persons receiving corticosteroid therapy, which depresses the inflammatory response, should also be excluded from the study. Fourth, a strong immediate response (20 minutes) at the reaction site may yield a false negative delayed reaction. Hence, subjects with a strong immediate response should be excluded from consideration in the analyses of the data (A11.2).

Trouble Shooting:

There are problems that arise with determination of skin test frequency within a population. The first problem is associated with the shelf life of immediate skin test reactions. The shelf life of reconstituted prick allergenic extracts is 18 months in 50% glycerine when stored at refrigerated temperatures. Therefore, for accurate determinations of immediate skin test reactivity in test and control populations, one must test both populations with the Same Lot of allergenic extracts within 18 months. It is not advisable to change lots of allergenic extracts during the course of the study. There is considerable variation in the allergenic potency of immediate skin test reagents when different lots of the same allergic extract are compared.

Although the prick test is considered to be a safe, rapid method for determining atopic status, one must be aware that systemic allergic reactions may occur in a small number of individuals. Therefore, a physician should be available in an emergency. Moreover, the skin testing facility should be equipped with a drug box containing the necessary resuscitation equipment and aqueous adrenalin (1:1000 V/V).

The serological reactivity of some delayed skin test reagents is not well standardized (i.e. SK/SD, Trichophyton and Candida). In this case, it is necessary to determine the concentration necessary to evoke delayed hypersensitivity reactions in number of normal controls prior to the start of the population study.

The lack of antigen standardization creates another problem. Some individuals will have massive delayed hypersensitivity reactions to low doses of SK/SD (4U/IU). These reactions include induration greater than 40 MM, sloughing of the epidermis and swelling of the entire forearm with associated joint pains. Although this problem is not serious, it does cause a great

degree of apprehension among other participants in the study. Should these accelerated reactions occur, a physician should be consulted and the reaction site covered with corticosteroid impregnated tape.

With respect to potency and shelf life, the same problems apply to the delayed skin test reagents that were outlined for the immediate skin test reagents. Care should be taken to insure that both the test and control population are tested with the same lot of antigen within the shelf life of the reagent.

There are problems associated with the determination of positive skin tests. To abrogate the variability in actually reading the positive skin tests, it is necessary to insure that the same individual(s) read the skin test during the course of the study. It is also necessary to make a prior criterion for a positive skin test, in terms of the size of the wheal or induration, for each antigen before initiating the study. Obviously, this criterion will depend on the sensitivity of the person reading the skin test.

Interpretation

Patterns of immediate skin test reactivity is difficult to interpret. In some industries, the more allergic individuals are forced out of the work environment. Hence, there is a survivor working population which will have overall patterns of immediate skin test reactivity which are lower than a control population from the same geographic area. Moreover, some individuals may have positive skin tests to specific allergens but no clinical episodes of asthma associated with exposure to the same allergens used in the skin tests.

The concept of a survival population is difficult to establish because of the limited number of individuals working in an industry. However, comparison of the skin test frequencies in: (a) control population; (b) currently working; and (c) non-working people who left the industry, should establish whether atopic sensitivity played a role in the decision to leave the industry, and whether occupation related allergenic activity was observed in the non-working group.

The relationship of positive skin tests to occupation-related allergens and occupation-related asthma must be confirmed. This can be achieved by demonstrating a strong correlation between a positive skin test to occupation-related allergens and a history of occupational asthma provoked by the same agent.

The presence of anergy to delayed skin test with a population can be assessed by several techniques as described by Spitler (All.2):

1. Positive responses to fewer than two of the five skin test antigens in an individual.
2. The total sum of induration to all five skin test antigens is less than 10 MM.

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All.3 Norman, PS, Lichtenstein, LM and Ishizaka, K: J. Allergy & Clin. Immunol., 1973, 52:210.

SKIN TEST - PRICK TEST

STUDY ID# /___/ /___/ /___/ /___/ /___/ /___/

COMMON ATOPIC ALLERGENS	DIAMETER (mm) ERYTHEMA	WHEAL
Ragweed		
Timothy grass		
Feathers		
Oak		
Cat		
Rat		
Other		

FUNGAL ANTIGENS	DIAMETER (mm) ERYTHEMA	WHEAL
Asper. fumigatus		
Penicillium sp.		
Mucor sp.		
Cladosporium sp.		
Alternaria sp.		
Rust		
Smut		
Other		

INSECTS	DIAMETER (mm) ERYTHEMA	WHEAL
Mites mixed		
Beetles mixed		
Weevils		
Other		

GRAIN	DIAMETER (mm) ERYTHEMA	WHEAL
Wheat durum		
Wheat spring		
Barley		
Corn		
Rye		
Oats		
Sunflower seed		
Small seeds mixed		
Soybean		
Other		

AIRBORNE DUST	DIAMETER (mm) ERYTHEMA	WHEAL
Wheat durum		
Wheat spring		
Barley		
Corn		
Rye		
Oats		
Sunflower seeds		
Other		

SETTLED DUST	DIAMETER (mm) ERYTHEMA	WHEAL
Dust I		
Dust II		
Dust III		
Other		

SKIN TEST - INTRADERMAL

DELAYED 48 HOURS

	PPD		MUMPS		CANDIDA		SK-SD		TRICHOPHYTON	
	(Diameter mm)		(Diameter mm)		(Diameter mm)		(Diameter mm)		(Diameter mm)	
STUDY ID #	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL	ERYTHEMA	WHEAL
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