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American National Red Cross Blood Programme
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BLOOD PROGRAM DIRECTIVE

Section: Medical

Subject: Testing for Hepatitis Associated Antigen (Au)

Purpose: To provide information regarding testing for the Hepatitis Associated Antigen

The testing of all donor blood for HAA shall be routine in all Red Cross Blood Centers. No unit of blood or components shall be released until the Hepatitis Associated Antigen test has been completed except in emergencies, and in full compliance with paragraph 73.3004 (f) and with paragraph 73.3005 (e) of Title 42, PHS regulations, Part 73.

General Information

National headquarters has evaluated a variety of test systems for determining the presence of the Hepatitis Associated Antigen (HAA), considering four factors: (1) sensitivity, (2) simplicity, (3) availability and (4) cost.

The counterelectrophoresis method is recommended as the most practical test for HAA by blood-collection agencies at the present time. New procedures for the detection of HAA are being developed. It is possible that the methods now recommended may become obsolete within 18 months.

The procedure selected for the American Red Cross Blood Program is Spectra's Hepascreen Immunoelectrophoresis System (counterelectrophoresis). Supply and equipment items consist of (1) power source with built-in electrophoresis tray and viewer, (2) prepoured agarose gel plates, (3) buffer solution, (4) capillary pipettes, (5) HAA positive control serum and (6) anti-HAA serum. All the above material, except capillary pipettes, will be supplied by Spectra. The capillary pipettes (ARC # 348897) can be obtained through normal supply channels from the General Supply Office.

The Spectra system will be used by all centers which have not been routinely testing all donor bloods by the counterelectrophoresis method for Hepatitis Associated Antigen before February 1, 1971. Those centers desiring to continue using test systems in use prior to February 1, 1971, must apply for approval of their test system from the National Medical Director. The application must include a sufficiently detailed description of all items of equipment, specifications of agarose, type of buffer, pH, time of run, etc. Only licensed anti-HAA reagents may be used.

Work Area

A designated work area (used for no other purpose) must be provided in each center, preferably in or adjacent to the laboratory. The area must be separated by walls or partitions (not necessarily to the ceiling).

- A floor space of 6' x 6'4" is required for each two power units. This is to include bench space of 2' x 6'4". Centers using more than two units need proportionately larger space. Laboratory stools need to be provided.

- Electrical outlets need to be available for each power unit and a fluorescent lamp.

- Hand washing facilities should be available nearby.

- The inner room must have a waste receptacle with plastic liner for discard of contagious material.

- Gowns should be worn in the work area, but left there and never worn elsewhere. Gowns must be autoclaved before being laundered in the usual manner.

- Equipment and consumable supplies used in the work area should not be removed until properly disinfected.

Storage of Material and HAA Positive or Suspected Positive Units

All HAA testing kits that are unopened may be stored according to the manufacturer's directions in any suitable refrigerator including a walk-in where they can be separated from blood, blood components, or derivatives.

Opened HAA testing kits cannot be stored with blood, blood components, or derivatives. They must be kept refrigerated between 2° and 8° C. and can be stored in same refrigerator as typing sera.

Storage of Blood

Units of blood which have been found to be positive for HAA, or suspect units, must be wrapped in plastic bags closed with rubber bands and appropriately labeled. They can be stored in refrigerator with typing sera (NOT IN ANY REFRIGERATOR CONTAINING BLOOD OR COMPONENTS) prior to shipment to national headquarters.

Performance of Test

1. Follow manufacturer's directions.

2. Wear disposable gloves (ARC # 343950).

3. Large amounts of buffer may be prepared ahead of time and stored up to one week in the refrigerator. Daily amounts of buffer must be warmed in a 37° water bath to redissolve any barbital which has precipitated. Discard buffer after use.

4. Place positive control in upper left and lower right on each plate.

5. Turn off viewer lights during test run.

6. Wipe electrodes at end of each test run.

Interpretation of Test

A positive test is indicated by a sharp precipitin line parallel to the row of wells. Although the Spectra system has a built-in viewer, reading of the precipitin line is improved by using indirect lighting (a shielded fluorescent desk lamp) against a dull black background, and therefore all tests must be read in this manner. Before reading, the back of each plate should be wiped to remove moisture, lint and fingerprints.

Possible Sources of Error in Test Interpretation

- Excessive heat during electrophoresis may produce artifacts due to drying of the agar or denaturation of serum proteins.
- Anti-beta lipoprotein present in some sera may react with beta lipoprotein in the serum under test.
- Abnormally high levels of serum albumin can interfere in testing.
- Lipids in the donor serum may migrate during electrophoresis and obscure reading of positive reactions or be misinterpreted as a positive result.

Recording HAA Test Results

Neither Laboratory Form 4573 nor anything else to be used elsewhere can be taken into the work area used for the HAA testing;

therefore:

- Results of all HAA testing should be maintained in a notebook or "log book" which remains in the HAA work area.
- Daily, a routine should be established whereby the first and last number of the center and each mobile collection is recorded on a sheet of paper and taken into the HAA work area;

  e.g.: 23F09653 - 23F09681

  23G68740 - 23G68823; 68825 - 68971 etc.
- The log book must be kept in the HAA testing work area and must contain the date of test and the lot numbers of the antigen, antibody and agarose plates. The person or persons performing the HAA tests must sign the daily record in the "log book," as well as sign the appropriate column of Forms 4573.

Suggested Format for Log Book

Anti-HAA	Lot #A113
Antigen Control	Lot #202-1
Plate Number	
Date	
Technologist	

Whole Blood Number
1. 23F07653 - 681
2. 23G68740 - 768
3. 769 - 797 - #7 pos. - (23G68775)
4. 798 - 826 - #68824 missing
5. 827 - 855

- To avoid confusion when an occasional sample is missing, the corresponding wells may be left empty on the agarose plate. However, a mark should be made on the back of the plate with a felt tip pen under the wells which will not be filled to indicate that the sample is missing.

- If the suggested format is used, plates can then be labeled in simple numerical sequence - 1,2,3.. etc. When positive tests are noted, the position on the plate - e.g., well #7 - should be recorded as well as the corresponding whole blood number. The identification of reactive samples should be confirmed by repeating the test on a sample attached to the unit (the permanently attached pilot tube or segment). The label of this unit should then be defaced by boldly marking it "HAA reactive." This system of rechecking positive tests from a sample attached to the container itself will also help to insure that HAA reactive units are not accidentally distributed for transfusion. When the sample is proved reactive, all laboratory tubes or pipettes exposed to the sample must be treated with sodium hypochlorite.

- At the completion of testing, the HAA reactive samples must be noted on the "daily list." And later, the results can be recorded on Form 4573, page 2, in the column between "Isoagg titer" and "Remarks." The column should be labeled "HAA tests" and separate

entry(R, reactive or NR, nonreactive) made for each sample tested. Reactive results recorded on Form 4573 should be carefully rechecked for clerical accuracy. The slip of paper listing the samples tested that day should then be returned to the HAA test area. The whole blood number of reactive samples should again be rechecked with "HAA test results log book" before discarding the "daily list."

Labeling

All units nonreactive for HAA will be so labeled. DBS has recommended that the statement on the label be "Nonreactive for HAA by Counterelectrophoresis." Suitable labels will be provided when available. In the meantime, the stickers supplied by Spectra may be used. No unit of blood can be released until the HAA test has been completed except in extreme emergencies and the hospital receiving the blood must be so notified. A tie tag with lettering "HAA TEST - NOT DONE" - must be attached to each unit.

Disposition of HAA Positive Units

Although HAA positive units of blood cannot be used for transfusion purposes, they may serve a very useful purpose in investigating various aspects of the hepatitis problem. It is well known that the "hepatitis antigen" is not a single entity but is composed of a number of subgroups which have not been completely identified. Therefore, it is important for national headquarters to establish a library of antigenic variants. Dr. Tullis has reported a significant reduction in posttransfusion hepatitis by a transmembrane washing procedure on thawed, deglycerolized, previously frozen red blood cell (JAMA 214: 719-723, 1971). Dr. Meryman of the Red Cross Research Laboratory has considerable research interest in this field. It has been found that following fractionation of HAA positive plasma, a high concentration of anti-HAA antibody may be present in Fraction II. Anti-HAA is potentially useful in the treatment of hepatitis.

For the above reasons, ALL REACTIVE OR SUSPECTED UNITS, together with satellite packs and attached pilot tube or segments should be shipped in standard Red Cross shipping containers with ice (9 lbs.) to

American National Red Cross(Laboratory)
1730 E Street, N.W.
Washington, D.C. 20006

HAA reactive units must be placed in a plastic bag, closed with rubber bands and isolated in an appropriately labeled carton prior to shipment. The bag used for icing shipments (ARC # 341281) may be used. The pilot tube or segments will be used by the Reference Laboratory (Hepatitis Section) for confirmation studies and the results reported to the center.

Units should be shipped, weekly - on Tuesdays, Wednesdays or Thursdays - by prepaid Air Freight. Arrangements have been made with the Airborne Freight Corporation. The local representative of Airborne Freight should be contacted. Specify "ROUTINE PICKUP" service, when the cartons are ready. The outside of shipping container should be labeled - "BLOOD FOR RESEARCH."

Airborne Freight Waybills (see attachment #2) can be obtained from your local representative. Waybills should be marked "Blood for Research." Until a new form (Hepatitis Associated Antigen, Form 5707) is available, the Rare Donor Form 5631 in duplicate must accompany each unit of blood.

Three copies of Form 5478 labeled "RESEARCH" will accompany each shipment. One copy will be returned to the center, one copy will be sent to the Red Cross Blood Research Laboratory in Bethesda and one copy will be retained at Reference Laboratory.

Disposition of Contaminated Materials

Sodium Hypochlorite Solution:

A 5% solution of sodium hypochlorite can be purchased locally for use in disinfection of waste materials connected with HAA testing. This solution is equivalent to full strength "Clorox" It has been suggested that centers contact a wholesaler to arrange for large scale procurement - i.e., gallon/drum quantities. Enameled or plastic pails or garbage cans are suitable for use with hypochlorite solution; non-enameled metal containers must not be used since they will be corroded by sodium hypochlorite solution. Used solution must be discarded. PROTECT HANDS WITH GLOVES WHEN HANDLING HYPOCHLORITE.

Liquid Waste:

Unless prohibited by local ordinance (check with local health department), liquid waste may be discarded in any laboratory sink drain or toilet after it has stood overnight in a 2.5% hypochlorite solution. This concentration may be achieved by mixing equal volumes of 5% sodium hypochlorite with buffer or with HAA reactive sera - or - by adding appropriate amounts of sodium hypochlorite powder to the liquid waste (e.g., - 16.25 gms. per 650 ml buffer).

Solid Waste:

All solid waste material associated with HAA testing will be disposed of in one of two ways:

1) Soak overnight in 5% sodium hypochlorite solution (special handling necessary for capillary tubes, see below); after 12 hours of soaking, remove waste from solution and discard with routine laboratory waste. Sodium hypochlorite solution must be discarded after each use. Uncoated metal containers must not be used since they will be corroded by the sodium hypochlorite solution. Enameled or plastic pails or garbage cans are suitable.

2) Wrap solid waste carefully, label, and incinerate daily. The wrapping must be a double bag of impervious plastic which can be tightly sealed. Seal must not be removed before incineration. Label should indicate to handlers that the package contains infectious material.

Capillary Tubes:

Unless incineration is available, each capillary tube must be placed upright in a solution of 5% sodium hypochlorite immediately after use. A plastic or glass drinking glass of the appropriate size may be used for this purpose. Dimensions of the container must be such that the capillary tubes remain upright and are completely submerged in the sodium hypochlorite solution. Allow capillary tubes to soak overnight and then discard solution and tubes.

Personnel Precautions

Since the HAA is considered contagious, adequate precautions (use of gloves, ARC stock # 343950, no mouth pipetting) must be taken.

The area in which HAA testing is being done must be disinfected after completion of each day's testing, using a disinfectant such as 1:2500 solution of Roccal (Winthrop). Soak racks, clean countertops, and clean floors with dilute sodium hypochlorite. (Dilute 5% hypochlorite solution 1:8 (.6%))

All paid staff personnel involved in direct contact with blood or its components in collection (hemoglobin and hematocrit tests, phlebotomy, sealing segments, filling pilot tubes, etc.), processing and distribution will be tested every 6 months for the presence of HAA. All new and present employees will be tested and, if a reactive result is obtained, referrred to their personal physicians. Personnel with reactive HAA tests, unless diagnosed as having overt hepatitis or in the prodromal stages of hepatitis, may continue to be employed in their present positions except those individuals directly involved with the actual phlebotomy or performing hemoglobin determinations prior to phlebotomy. Dated results of all tests (reactive or nonreactive) must be recorded in individual personnel files. This record should include laboratory studies by private physician on HAA reactive personnel.

Letter to Donors with Positive HAA Test

A letter, prepared with advice of legal counsel at national headquarters, will be sent to all HAA reactive donors. Any changes in the attached letter must be approved by national headquarters prior to use. For medico-legal reasons, any unit of blood found to be reactive to HAA by any center, or reported by hospital receiving unit, must be considered as reactive, although the unit may be reported as nonreactive at national headquarters' laboratory.

Registration of HAA Positive Donors

A master list of all HAA positive donors in addition to any donors on each center's "Hepatitis Suspect List" will be compiled by national headquarters and will be made available to all centers.

Proficiency Testing and Quality Control

1) Periodically each center will receive unknown specimens from national headquarters for testing. These samples must be tested and results submitted to national headquarters within 72 hours.

2) Each center will occasionally be requested to furnish national headquarters 50 to 100 sera from HAA negative units for testing.

Training

Training approved by National Medical Director is mandatory for all personnel involved in HAA testing.

Hepatitis Testing for Other Blood Collecting Agencies

For medico-legal reasons, no Red Cross center should make any commitment to screen units of blood or patients' samples for the presence of HAA for any hospital or other blood-collecting agencies.

All questions pertaining to the Hepatitis Testing Program should be referred to the National Medical Director.

NOTE: Whenever local laws are at variance with any portion of this directive, the local laws will prevail.

Dear _____:

I am writing to you in connection with your recent blood donation and first want to express our appreciation for your unselfish and sincere desire to help others.

Our Blood Center is now testing all blood for the possible presence of a hepatitis virus. The reason for this is that the transfusion of blood carries a risk of causing hepatitis in the patient receiving the blood. You may be aware of this fact since the problem of serum hepatitis has been widely publicized by the news media in recent months.

Tests on the unit of blood which you generously donated indicated the presence of a hepatitis agent. While this should not alarm you since it does not necessarily indicate any problem with your personal health, we suggest that you notify your personal physician.

Since the test indicates that your blood is potentially capable of causing hepatitis in a recipient you should not serve as a blood donor.

We realize that it may be difficult for you to understand completely why you cannot again be accepted as a donor. If you desire further clarification, you may want to discuss the matter with your personal physician or the medical director at the Red Cross.

Even though you cannot be a blood donor there are several ways you can help assure that there is an adequate blood supply for those in need. We hope you will persuade your family and friends to become regular volunteer donors. You can also help by recruiting donors where you work and in the organizations to which you belong.

We cannot thank you enough for your cooperation with the Red Cross Blood Program and hope you will continue to support the Program in the future.

Attachment #2
to BPD 4.29

AIRBORNE FREIGHT CORPORATION

COLMAN BLDG. – SEATTLE, WASH. 98104

48790

SHIPPER	CONSIGNEE
Your Center	American National Red Cross
STREET	STREET
	1730 E Street, N.W.
CITY	CITY
	Washington, D.C. 20006
DATE SHIPPED Date	Att: Mrs. Ni

TARIFF DESTINATION

SCALE NUMBER | COMMODITY

CHARGES - CHECK ONE: PREPAID [V] COLLECT [ ]

FREIGHT CHARGES

A. F. C. PICK UP

A. F. C. DELIVERY

EXCESS VALUE

INSURANCE

ADVANCES/CHARGES ORIGIN

ADVANCES/CHARGES DESTINATION

C.O.D. ⇒

C.O.D. FEE ⇒

PIECES	DESCRIPTION OF PIECES AND CONTENTS	WEIGHT	RATE
#	Human Blood (For research)	approx.	
	SPECIAL SERVICES REQUESTED		
	WEIGHTS SUBJECT TO DIMENSIONAL CORRECTION X X =	TOTAL	

AFC ROUTING	RECEIVED BY AIRBORNE FREIGHT AT:	DECLARED VALUE	INSURANCE REQUESTED BASED ON DECLARED VALUE SHOWN	U.S.	U.S.
	SHIPPER'S DOOR [V] AIRBORNE TERMINAL [ ]	$ 50.00	YES [ ] NO [ ]	$	$

SHIPPER'S SIGNATURE	CONSIGNEE'S SIGNATURE	TOTAL CHARGES
X	X	

DATE—TIME RECEIVED	FORWARDER'S SIGNATURE	DATE—TIME DELIVERED	4879073

NON NEGOTIABLE AIRBILL SUBJECT TO CONDITIONS SET FORTH ON REVERSE SIDE HEREOF.

O-202 REV. 7-70

1 - SHIPPER'S COPY