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TRANSFUSION-TRANSMITTED VIRUSES STUDY

PROTOCOL OPERATIONS MANUAL

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I. ADMINISTRATION

I-A. Background

Introductory Summary

PILOT PROGRAM

The Transfusion-transmitted Virus Study Group was organized on July 1, 1974, under terms of an 18-month contract, to organize a prospective definition of trends in incidence and etiology of transfusion-associated hepatitis. Donor characteristics, both informational and laboratory, are to be evaluated for risk of the donation to recipient. Serum from all donors, recipients, and controls is stored to permit evaluation of future screening tests.

The Group met July 1 and 2, 1974, to devise a protocol and procedures. The source of the Study's populations is from patients cross-matched for potential blood transfusion because of scheduled surgery. As a result, recipient and control populations (with the exception of cardiac surgery patients, for whom adequate numbers of non-transfused controls are lacking) are probably as closely matched as any transfused - non-transfused groups can be.

During the first six-month period (July-December, 1974), emphases at the investigative centers were on recruiting and training staff, devising donor and patient specimen collection procedures, and testing feasibility of various methods for screening, enlistment, and enrollment. The coordinating center focused upon uniformity in eligibility criteria at the three investigative centers, and on the design of both forms and computer data acquisition programs suitable for screening, enlistment, and enrollment data. During the second six-month period (January-June, 1975), emphasis has been upon standardizing data collection procedures for routine and "special" (suspected hepatitis) follow-up data. A computer program for acquisition of follow-up data became available in late January, and has been used for all newly enrolled patients since that time. Unfortunately, considerable time has been required to code and build data files for the patients who had entered the study during its first seven months. Programs now exist to schedule patient follow-up for each center each week. Programs have also been formulated to identify missing data and for chronological display of all information pertinent to a patient's health status throughout the period of observation. During the last six-months of the pilot project (July-December, 1975) attention was focused upon data acquisition programs for routine donor information, donor laboratory data selected implicated and control follow-ups, a donor data display program, and computer programs for quality control procedures in the laboratory.

During the 37-week period from July 1, 1974 through March 16, 1975, the three investigative centers screened 1,982 patients who were potential transfusion recipients. There were 499 who met the eligibility criteria and consented to participate (i.e., they were enlisted). Of these, 407 (81.6 percent) were enrolled (i.e., continued eligibility and willingness to cooperate were reconfirmed two weeks after transfusion or surgery). Emphasis was placed on low volume recipients to permit the donor to be implicated as closely as possible (mean 3.8 units, median 3.0). The total period of patient follow-up is 10 months, so that an estimate cannot be made at present of the proportion likely to complete the entire 40-week program. For patients enrolled in the 11-weeks from January 1 through March 16, 1975, the completeness of routine follow-up was 80 percent. Thirty-seven of 407 patients had "special" follow-ups because of an event possibly requiring further evaluation for viral hepatitis. Delivery of 5.0 ml aliquots of donor and patient sera began November 1975, as directed by NHLI.

NEW PROGRAM

The fully operational project involving 4 investigative centers, over the next 5 years, is designed to identify at least 400 cases and 1,000 donors. The hepatitis cases will be characterized as to incubation period and etiology (either positively as type B hepatitis or negatively as "non-B for the latter). Donors will be particularly evaluated with regard to ALT level and risk, in view of preliminary evidence from UCLA. Emphasis will also be placed upon anti-HB_S risk in relation to non-B hepatitis, both for paid and unpaid donors, and for /ad and /ay subtypes (Goldfield, Amer. J. Med. Sci., in press).

RESEARCH GOALS

Broadly interpreting the National Heart and Lung Institute's concern about the problem of transfusion-associated viral hepatitis (RFP HB 74-14), the investigators wish to address themselves to the elements of the study that they feel summarize the longer range goals.

I. There is a need to establish prospective surveillance of the trends in incidence and changes in etiology of transfusion-associated hepatitis in the United States. There are, however, several variables that influence rate, so that the method of study and/or the way in which rate is expressed, must be sufficiently standardized, that comparability is achieved. Unless the scope of transfusion-associated hepatitis is adequately monitored over a sufficient period of time, it will be very difficult, or even impossible, to know the amount of effort the problem justifies, or the impact of measures introduced hopefully as preventatives, but confounded in their evaluation because they are adopted simultaneously or in quick succession. When these measures are very expensive, such as washed and frozen red cells, the magnitude of the problem is obvious.

II. There is a need to establish a bank of sera from a group of donors and recipients of sufficient size and well enough characterized to provide almost immediately an accurate evaluation of the ability of newly introduced tests to accomplish the following:

1. It is well-known that even the best techniques for screening for HB_sAg do not at present prevent occasional cases from occurring (1). As new procedures become available (2,3), we must find out what proportion of HBV-positive donor sera could be documented.

2. Even without new laboratory techniques, donors to cases documented by prospective study as type B in etiology may have some non-serologic characteristic that identifies them as a high risk group. Thus, Egoz and co-workers (4) showed that Israeli donors born in North Africa were more likely to be carriers than European-born Jews long before HB_sAg testing showed that they had showed a higher risk attached to prior transfusion at any time in life, a finding subsequently confirmed in terms of carrier rates by Lewis et al (6).

3. There is strong evidence for presently uncharacterized agents as a cause of human hepatitis, especially transfusion-associated hepatitis (7-9). If procedures for diagnosis of "non-A, non-B" hepatitis become available, we shall need to know quickly the proportion of presently undiagnosable cases due to this etiology, and the carrier rate among donors.

4. Careful characterization HBV-negative donors in relation to HBV-negative cases again may point to a demographic or other personal characteristic of help in screening, or to a non-specific test such as a serum enzyme assay.

In fact, there is a third long-range concern implicit in the study being considered, that could be even broader in its implications for transfusion therapy. On occasion, neoplasia may be communicated through transfusion of whole blood or some blood components. One mechanism would be the transfer of neoplastic cells themselves, although immunologic defense mechanisms would presumably be able to protect the recipient against such "foreign" cells in the vast majority of instances. There is, however, increasing evidence for oncogenic viruses as the basis for at least some human cancers. Considering the long latent period that would be suspected from experimental work in animals any relationship may well have gone unnoticed previously. If a test for a human oncogenic virus is found, a well-documented set of data and sera from donor, recipients, and controls would be invaluable as the basis of a study that in fact would be prospective (i.e., the persons considered in the numerator and denominator were defined prior to the development of characteristic in question). In addition, although there is no evidence for that at the present time, there has been some attention to the possibility that some slow viruses responsible for degenerative diseases of the nervous system could be transferred in this way (10). These considerations prompted the investigators to constitute themselves as the Transfusion-transmitted Viruses Study Group, and to suggest to NHLI that they retain sera from all donors, recipients, and controls, rather than those alone who evidenced clinical or subclinical hepatitis.

There are a number of variables known to influence the rate of transfusion-associated hepatitis:

1. The amount of each etiologic form of viral hepatitis in the community at the time (11), which may be expressed in terms of transfusion-associated disease through asymptomatic infections in persons serving as blood donor, or as disease acquired through other mechanisms by individuals who happen to have been transfused also.

2. The previous experience of a community with hepatitis virus(es) capable of producing a long-term carrier state, even when subsequent transmission is at a very low level (12).

3. The current and previous experience of the portion of the community from which blood donors are derived.

4. The number of units of blood given, which is not related to attack rate in an arithmetically linear way (4,13).

5. The immune status of the recipient population through previous experience by other mechanisms (14).

6. The definition of viral hepatitis, which is a result of the method of follow-up and of the definitions used, which have varied widely (Seeff, unpublished data).

7. The effect of various methods of prevention that may be used to varying extents at any given time or from time to time.

The present study has attempted to take these various factors into account. It was demonstrated in the Pilot Study that the investigative centers can recruit non-recipients and follow them as successfully as recipients. It has also been shown that non-cardiac patients will participate if blood collection is made at their home if they have no reason because of personal health to go to the hospital. The prior experience with HBV can be defined for both donors and recipients (by HB_sAg and anti-HB_s testing), who are characterized also with respect to a number of other factors through special follow-up.

REFERENCES

1. Goldfield, M.; Black, H.C.; Bill, J.; Srihongse, S.; Pizzuti, W.: The Consequences of Administering Blood Pretested for HB_sAg by Third Generation Techniques: A Progress Report. Amer. J. Med. Sci. (in press).
2. Hoofnagle, J.H.; Gerety, R.J.; Barker, L.F.: Antibody to Hepatitis-B-Virus Core in Man. Lancet 2: 829, 1973.
3. Kaplan, P.M.; Greenman, R.L.; Gerin, J.L.; Purcell, R.H.; Robinson, W.S.: DNA Polymerase Associated with Human Hepatitis B Antigen. J. Virol. 12: 995, 1973.
4. Egoz, N.; Brachott, D.; Mosley, J.W.; Yekutiel, P.: Viral Hepatitis in Israel: Transfusion-associated Hepatitis. Transfusion 12: 12, 1972.
5. Bar-Shany, S.; Naggan, L.; Wolpiansky, N.: Hepatitis-associated Antigen in 10,000 Israel Blood Donors. Israel J. Med. Sci. 3: 1, 1972.
6. Lewis, T.L.; Alter, H.J.; Chalmers, T.C.; Holland, P.V.; Purcell, R.H.; Alling, D.W.; Young, D.; Frenkel, L.D.; Lee, S.L.; Lamson, M.E.: A Comparison of the Frequency of Hepatitis-B Antigen and Antibody in Hospital and Non-hospital Personnel. N. Engl. J. Med. 289: 647, 1973.
7. Prince, A.M.; Brotman, B.; Grady, G.F.; Kuhns, W.J.; Hazzi, G.; Levine, R.W.; Millian, S.J.: Long-incubation Post-transfusion Hepatitis Without Serological Evidence of Exposure to Hepatitis-B Virus. Lancet 2: 241, 1974.

REFERENCES

8. Feinstone, S.M.; Kapikian, A.Z.; Purcell, R.H.; Alter, H.J.; Holland, P.V.: Transfusion-associated Hepatitis Not Due to Viral Hepatitis Type A and B. N. Engl. J. Med. 292: 767, 1975.

9. Mosley, J.W.; Redeker, A.G.; Feinstone, S.M.; Purcell, R.H.: Etiologic Studies of Multiple Attacks of Acute Viral Hepatitis. (Submitted).

10. Herzberg, L.; Gibbs, C.J., Jr.; Asher, D.M.; Gajdusek, D.C.; French, E.L.: Slow, Latent, and Chronic Viral Infections of the Central Nervous System. In Transmissible Disease and Blood Transfusion, edited by T.J. Greenwalt, G.A. Jamieson. Grune & Stratton, New York, 1974. p. 14.

11. Mosley, J.W.: Epidemiologic Implications of Changing Trends in Type A and Type B Hepatitis. In Hepatitis and Blood Transfusion, edited by G.N. Vyas, H.A. Perkins, and R. Schmid. Grune & Stratton, New York, 1972, p. 23.

12. Cherubin, C.E.: Risk of Post-transfusion Hepatitis in Recipients of Blood Containing S.H. Antigen at Harlem Hospital. Lancet 1: 627, 1971.

13. Allan, J.G.; Sayman, W.A.: Serum Hepatitis from Transfusions of Blood. JAMA 180: 53, 1962.

14. Cherubin, C.E.; Purcell, R.H.; Lander, J.J.; McGinn, T.G.; Cone, L.: Acquisition of Antibody to Hepatitis B Antigen in Three Socioeconomically Different Medical Populations. Lancet 2: 149, 1972.

REFERENCES

15. Rodgerson, D.O.; Osberg, I.M.: Sources of Error in Spectrophotometric Measurement of Aspartate Aminotransferase and Alanine Aminotransferase Activities in Serum. Clin. Chem. 20: 43, 1974.

I-B. Facilities and Resources

1. USC

The coordinating center is located in the Hepatic Epidemiology Laboratory. Its staff is part of the Department of Medicine, University of Southern California School of Medicine. The facility is part of the Liver Service, Rancho Los Amigos Hospital. The 86 beds of the Liver Service, with some 700 to 800 admissions annually for viral hepatitis, make it the largest unit of its type in the country.

The Hepatic Epidemiology Laboratory occupies five offices and three laboratory rooms, with approximately 1600 square feet of space. It has had extensive experience with serologic testing for HBV in both patient and outside population groups, and with the development of panels for standardization of HB_sAg procedures. It served as the coordinating center for the NHLI-sponsored study of hepatitis B immune globulin in the treatment of fulminant type B hepatitis, and is the coordinating center for a newly initiated study of corticosteroid therapy of acute hepatic failure.

The coordinating center receives statistical and computer support from the Biostatistics Laboratory, Department of Medicine, under the direction of Dr. John M. Weiner. The Biostatistics Laboratory has had extensive experience with cooperative studies of therapy in heart disease and cancer. The transfusion-transmitted viruses study has benefited greatly from the ready availability of these programs as prototypes.

The investigators use the General Electric Time-Sharing System for computer entries, storage, and analysis of the data. This system, one of the largest in the world, provides access by means of a local telephone connection in all major cities of the United States. Each unit, as well as the coordinating center and NHLI, can have access to on-line data through terminals with telephonic connectors.

2. UCLA

The University of California, Los Angeles (UCLA) Center for the Health Sciences is a general hospital with 900 beds providing services in each of the major medical and surgical subspecialties. Eighteen thousand, six hundred patients are hospitalized anually in this institution. The UCLA Blood Bank processes 12,000 units of blood anually, utilizing both paid and volunteer blood donations.

The experience and facilities of the UCLA Center for the Health Sciences and members of the Department of Medicine have been and will continue to be available to insure the efficient development of this study. The Medical Outpatient Clinic, which consists of two waiting rooms and forty examination rooms, is available for the outpatient evaluation of patients in this study.

The Department of Medicine maintains a full intern and resident house staff program, nurses, dieticians, intensive care units, and all appropriate facilities for the proper monitoring and care of patients entered into this program. Members of the liver study group will assume responsibility for the follow-up of patients entered into this program, and medical supervision of those who develop viral hepatitis. Whenever consultative services are needed, the major surgical and medical divisions are available to provide services, including gastroenterology, cardiology, hematology, pulmonary disease, nephrology, metabolism, neurology and pathology.

Laboratory tests will be conducted in the facilities of the Gastroenterologic Virology Unit. These facilities constitute six hundred square feet of laboratory space. The virology laboratory contains appropriate hoods, incubators, and freezers. For the performance of hepatitis B antigen assays, a gamma counter, counterelectrophoresis equipment, and microtiter complement fixation and hemagglutination equipment are available. Four minus-seventy degrees C. upright 15 cu. ft. freezers are available, as are standard refrigerated centrifuges, refrigerators and basic laboratory equipment. A walk-in minus-twenty degrees C. freezer and a walk-in minus-four degrees C. refrigerated room are also available.

The proximity of the Coordinating Center at USC to the Investigative Center at UCLA have facilitated the development of the protocol, forms and procedures by the Coordinating Center in permitting the ready testing of ideas in terms of their practicality. At the same time, the Coordinating Center's staff is readily available to assist in clarifications and problem solving.

3. St. Louis

Washington University Medical Center, including the Barnes Hospital Complex, has approximately 1,200 beds and 37,000 patient admissions annually on the Medcial, Surgical, OB/Gyn, ENT, Opthalmology, and Pediatric Services. Approximately 14,000 units of blood are transfused each year, the vast majority to patients of the Medical and Surgical Services. Blood is drawn entirely from volunteer sources and comes from a stable population of donors from the greater St. Louis area. Most of the blood is provided by the St. Louis Regional Blood Center of the American National Red Cross, which collects and dispenses approximately 150,000 units of blood per year. Dr. William Miller, Director of the Regional Center, and Dr. Lawrence Sherman, Director of the Blood Bank at Barnes Hospital, have arranged to provide the donor specimens for patients enrolled in the Transfusion-Transmitted Viruses Study.

To accomplish this, a 15 ml pilot tube is drawn at the time of collection from each donor, labeled, racked, and set aside for the duration of the shelf life of the unit. In this way, it is possible to identify and permanently store samples of every unit of blood given to patients admitted to the study.

Both donors and recipients are tested for HB_sAg by radio-immunoassay at the new 35,000 sq. ft. facility of the Regional Blood Center, American National Red Cross. Tests for anti-HB_s and for ALT of recipients and a subset of donors are conducted by the staff of Dr. Richard Aach. His laboratory is part of the Division of Gastroenterology at Washington University Medical Center, on the premises of the Barnes Hospital complex. Dr. Aach's laboratories occupy approximately 800 square feet, housing two International Centrifuges and a Nuclear-Chicago automatic gamma counter. A Project Coordinator, nurse-epidemiologist, technicians, secretary, and part-time clerk are located adjacent to the laboratories assigned for the Transfusion-Transmitted Viruses Study to insure close interaction of all personnel and staff.

Dr. Aach and his staff earlier completed successfully a 2.5 year prospective study of transfusion-associated hepatitis, and have been able to bring the benefit of that experience to the present study.

4. Houston

Baylor College of Medicine has a primary affiliation with two hospitals; Ben Taub General Hospital, a 500-bed facility, and Methodist Hospital, which has 1,000 beds. Both hospitals are multi-service institutions. In addition, Ben Taub Hospital has an extensive out-patient clinic. Thus far in the Transfusion-Transmitted Viruses Study, patients have come from Ben Taub Hospital because of the convenience of the arrangement with its blood bank, and the fact that the present program is a continuation of a previous study of transfusion-associated hepatitis. Approximately 10,000 to 12,000 donor units are used each year at Ben Taub Hospital. Consultation and follow-up of patients is available through Dr. David Graham of the Gastroenterology Service.

Laboratory and office space is located in the Department of Virology and Epidemiology, where 1,640 sq. ft. have been set aside for the study. The laboratory is divided into nine individual glass-enclosed modules and has its own air conditioning and ventilator system to minimize cross-contamination with other virus laboratories in the department. It is fully equipped to conduct radioisotope studies.

The laboratory contains a 300-sample Nuclear-Chicago autogamma counter, one ultralow (-90 degree C.) temperature freezer, two -20 degree C. freezers, three +4 degree C. refrigerators, an RC-3 Sorvall, an IEC PR-6 refrigerated centrifuge, a Beckman L-40 preparative ultracentrifuge, a Sterigard negative pressure hood, a fluoroscopic microscope room, and an office. Available in the Department and readily accessible are preparative ultracentrifuges, an electron microscope, scintillation counters, LKB balances, a refractometer, liquid nitrogen freezer storage, another 300-sample Nuclear-Chicago autogamma counter and other ancillary equipment. Other facilities include a centralized location for washing and sterilizing glassware, autoclaves for decontamination, a walk-in refrigerator, and freezer lockers for storing sera.

5. New York

The Lindsley F. Kimball Research Institute is a part of the New York Blood Center, the largest blood collection site in the world (500,000 units of blood per year), and one of the largest blood research facilities in the U.S.A. Research at the Institute is supported by approximately 30 research grants and contracts from federal agencies and foundations; the current research budget approximates three million dollars per year.

The Laboratory of Epidemiology of the Lindsley F. Kimball Research Institute was established in 1973. Its main research direction is the epidemiology and prevention of viral hepatitis and other transfusion-associated diseases. The Laboratory occupies 4 offices and 3 laboratory rooms, with approximately 1,800 sq. ft. of space. Up to now, laboratory support for epidemiologic studies was provided by the Virus Laboratory (head: A. M. Prince, M D.). However, by November, 1975, a separate Hepatitis Testing Laboratory will be organized within the Laboratory of Epidemiology. Hepatitis testing will be supervised by Dr. Ben-Porath, previously Associate Professor and Director of the Virus Laboratory at Haifa University Medical Center in Israel.

From 1971-74 the Laboratory of Epidemiology conducted several large-scale population studies, particularly among blood donors, involving more than 30,000 people from various settings and areas in the greater New York metropolitan area and outside the U.S.A. This Laboratory, together with the Virus Laboratory, also served as the coordinating center for the NHLI-sponsored hepatitis B immune globulin trial in 15 dialysis centers. The present staff of the Laboratory consists of 8-10 highly qualified epidemiologists, field workers, and a systems-analyst. Computer support is provided by a central computer, IBM 370/125, and by Digital Equipment, PDP 11/70, PDP 11/10 and PDP 8E.

The study population for the proposed project will be drawn from patients treated at the New York Hospital-Cornell Medical College. The hospital is located two blocks from the New York Blood Center, and the director of the Blood Center, Aaron Kellner, M.D., is also Clinical Professor of Pathology and Director of the Blood Bank at the New York Hospital. The New York Hospital is one of the largest and most highly regarded hospitals in this area, with 1440 beds and 35,000 patient admissions per year. Approximatel 15,000 units of blood are transfused there each year; up to 90 percent of the blood is provided by the New York Blood Center. Arrangements will be made to prepare an additional 15 ml. pilot tube for all blood units supplied to the New York Hospital during this study. The Blood Bank of the New York Hospital has previously participated in a number of studies carried out by the New York Blood Center, including extensive follow-up of recipients of blood.

I-C. Organization and Administration

The protocol for the study and subsequent modifications in it emerged by concensus of the coordinating center staff and the participating principals from the three investigative centers. Each element of the protocol, and any modifications in it, have required the acquiescence of all, although the particular procedure in question at any given time did not necessarily represent the preference of all. This approach was adopted because any stipulation incompatible with the operative capacity of one center would clearly jeopardize the validity of the study. The basic approach to the questions to be answered and the materials to be obtained was evolved at the July, 1974 meeting and has been modified as necessary from subsequent experiences. The program was reviewed in September, 1974 (a conference call) and at meetings in October, 1974 (Los Angeles), November, 1974 (Chicago), January, 1975 (Houston), March, 1975 (Washington, D.C.), May, 1975 (San Antonio), and October, 1975 (St. Louis). One or more representatives of NHLI attended three of these meetings (Los Angeles, Houston, and Washington, D.C.).

The study recognizes that variations must be allowed in operative details from center to center. Similarly, some stipulations concerning type and use of personnel are left flexible to fit circumstances. Persons with key organizational and/or management responsibilities must be well-defined because of their responsibilities for understanding and interpreting procedures and answering for the adequacy and accuracy of the data.

Each center (including the coordinating center) has named a project coordinator other than the principal investigator. This person shall have immediate responsibility to the participating principal and have, as his or her primary function, the integration of all components into a smoothly operating unit. The project coordinator will handle routine problems, routine administrative contacts and be the person within the study structure aware of fiscal arrangements. The project coordinator at the participating center is the person contacted by the coordinating center concerning problems and priorities.

Each center has designated a data manager who is responsible for the editing of data, assembly of patient files and the computer.

Each center has designated a laboratory manager whom the Chief Technologist at the coordinating center will contact regarding discrepancies in quality control, incompatibilities in results, and problems concerning techniques.

Publication of reports involving data collected at all four investigative centers and correlated at the coordinating center will be published as a collective report of the "Transfusion-transmitted Viruses Study Group."

Title will come first, followed by authorship (TTVSG). A separate table will list the individuals. The first listing will go to the individual if a special contribution has been made. Collective contributions will be on a rotating basis and will be alphabetical. Any exception will be decided upon by a group vote. There will be no collaboration without group opportunity. The first name listed will receive reprint requests; this will give some recognition for special contribution. Each institution will list the principal investigator and up to four authors; names can change for work performed.

NHLI has initiated a contract with Flow Laboratories to provide a repository for the specimens collected for NIH. The manager of Flow Labs will check reported inventories against deliveries and check the adequacy of the 5 ml standard by random weighing of samples. Permission to use sera from the reserve shall be the decision of a Use Committee named by NHLI; participating investigators feel strongly that one person from each institution should be included because of the enormour effort made by each of the centers.

The Group urged NHLI to establish an Advisory Committee for the Study to which the Group can take problems for policy decisions. The adequacy of the number of accessions, the proportions of recipients and controls, new tests to be applied - all represent issues to be deliberated with an appropriate group of consultants, which has been appointed and is known as the TTVSG Advisory Board.

TASKS: COORDINATING CENTER

1. To have and maintain a manual of procedures fully reflective of both theoretical and practical modifications agreed upon during the interval and covering all aspects of the program.

2. To review, both routinely and as problems arise, the manual of procedures, and to revise promptly those portions which the participating principals and the NHLI Advisory Board agree should be changed.

3. To have and revise as appropriate a set of computerized data acquisition programs covering all aspects of the project, permitting prompt entry of information pertinent to patients and donors.

4. To provide each center upon their request with printout of 20 patients on a delayed overnight run.

5. To supply all forms and to revise them (with the data acquisition programs to which they are keyed) whenever sufficient number of changes justifies their expense.

6. To monitor each center on a monthly basis regarding all data information (status, trauma, infection, values, etc.); to double-check accuracy of information being stored in the computer.

7. To provide summaries of procedural progress at intervals of three months including pertinent information and the quarterly report.

8. As rapidly as is permitted by the amount of data accumulated, to add to the quarterly report analyses pertinent to the scientific questions being asked by the project.

9. To the extent permitted by budgetary considerations, to supply special analyses to requesting units.

10. To monitor various aspects of the data being accumulated, and to develop and test new uses or subprotocols that will enhance the value of the project without adding to the cost.

11. To provide suitable training material for orientation for new members of the staff of the investigative centers, and to hold at least one training session annually for project coordinators, data managers, and laboratory managers.

12. To carry out methodologic evaluations of laboratory procedures needed for appropriate functioning of the project.

13. To assume primary responsibility for the drafting of reports for the medical literature.

14. To maintain budgetary supervision for the investigative centers as subcontractors.

15 To carry out interim and final statistical analyses of trends and results as requested by the participating principals and/or the NHLI Advisory Board.

TASKS: INVESTIGATIVE CENTERS

1. To maintain an effective screening and enlistment program that identifies cross-matched patients prior to the time of potential transfusion.

2. To obtain an average enrollment each week of three recipients of transfusions (whole blood and/or single unit derivatives) and three controls who had been cross-matched but did not require blood.

3. To balance recipients to controls; if there is an abundance of controls, they should not be listed until the number of recipients is comparable.

4. To maintain routine follow-up of all recipients and controls with an assessment of health, of serologic status, and of biochemical parameters for viral hepatitis at intervals from the date of reference (transfusion, operation, or enlistment) of 4, 6, 8, 10, 12, 15, 18, 21, 24, and 40 weeks.

5. To obtain an assessment of the following laboratory parameters at enlistment, day of enrollment, and at each follow-up: serum alanine aminotransferase activity. Testing for HBsAg (AUSRIA, Abbott Labs) and anti-HBsAg (AUSAB, Abbott Labs) will be done by each center at weeks 0, 24, and 40.

6. To initiate special follow-up at weekly intervals, obtaining all pertinent information necessary for the usual evaluation in differential diagnosis, if viral hepatitis is suggested by symptoms, enzyme abnormalities, or serologic changes.

7. For each recipient with an episode evaluated as hepatitis, to select the first prior patient receiving the same number of units of blood from the same collection agency, and to carry out special donor follow-up in a manner entirely analogous to that for implicated donors. If at all possible, the nurse conducting the interview should not be aware of whether the donor is implicated or a control.

8. To obtain suitable information to identify each donor to each recipient through the various blood banks that are used. This must be done at least once a month.

9. To set aside and deliver to NHLBI at periodic intervals two aliquots of 2.5 ml each of serum or plasma from each donor, and the same from each evaluation of the patients. The rate of successful compliance shall be at least 90 percent of all specimens obtained from recipients and 100 percent on donors.

10. To set aside and deliver to the coordinating center at stipulated intervals an aliquot of 0.5 ml of each donor and patient sample for quality control determinations. The rate of successful compliance shall be at least 90 percent of all specimens taken. Subtyping on specific recipients and donors will also be done from this sample.

11. To select non-standard but promising procedures and to test specimens in order to provide information concerning its potential value in screening and diagnosis.

12. To carry out detailed follow-up, both informational and serologic, of closely implicated donors and a matched sample of non-implicated donors known as controls.

13. To test each donor specimen for alanine aminotranserase (ALT) to determine its potential usefulness in donor screening.

14. To computerize all data as soon as acquired.

15. To utilize standards and test panels (explained in Sec. VII) provided by the coordinating center laboratory, and comply with group decisions concerning standardization of procedures prescribed by the protocol.

16. To provide at least one representative to all meetings of the participating principals, and the appropriate person(s) to all training sessions.

17. Fill out Preliminary Report Form which is to go to coordinating center when first suspected of being a case.

18. Upon completion of study, to fill in a final evaluation form which is to be computerized.

19. Submit a quarterly financial report within two weeks of the close of any quarter to the coordinating center.

II. CLINICAL PROCEDURES AND FORMS

II-A. SCREENING & ENLISTING PATIENTS FOR STUDY PARTICIPATION

IDENTIFICATION OF PATIENTS

Selection of study patients generally is based on a review of blood bank records for patients cross-matched for actual or potential blood needs. In addition, one or more of the following screening procedures are used:

1. Review of operative schedules.
2. Review of admissions to medical wards.
3. Review of admissions to the emergency room.

The hospital charts of the patients selected from these sources are reviewed. The patient is eliminated from consideration if an item of disqualification appears in his medical record. Failure to disclose a reason for elimination means the patient must be interviewed.

A screening interview determines whether a patient who appears eligible on the basis of his chart review, is indeed suitable for the study. The interview should be designed to cover those items stipulated in the eligibility criteria and the questions that appear on Form 1 - Screening Interview and Enlistment Data.

The following criteria will be applied to all patients:

1. The patient must be typed and cross-matched.
2. The patient shall be 16 years of age or older. If patient is under the legal age of consent (18 years) his permission and that of his parents will be obtained.
3. The patient should come from a geographical location and living situation that make continued participation throughout the study as likely as possible.
4. A categorical exclusion of any malignancy is probably the most desirable procedure, but, in the event of difficulty in accessing a sufficient number of patients, it will be acceptable if the patient does not have any form of malignancy making survival for 40 weeks unlikely, or with a potential for spread to the liver, nor any medication that would alter the host response to infection, i.e., immunosuppressive chemotherapy.

If the additional information obtained from the interview indicates the patient to be ineligible, or if a patient otherwise eligible refuses to give his consent to participate, he is designated a "screen-only" patient.

Patients remaining eligible after screening are asked to participate in the study prior to surgery, regardless of whether they are subsequently transfused. At this time, a full discussion of the study must take place. The importance of the follow-up process, including the information and blood collection, must be stressed. Emphasis should be placed on the ability and willingness to complete the entire follow-up process in order that maximal value from specific segments may be obtained. The interviewer should assess the apparent interest in the study demonstrated by the patient. If the patient consents, he becomes "enlisted." A study identification number is assigned and the data are recorded on Form 1 - Enlistment Data.

The study identification number assigned at enlistment consists of a four-digit number representing the sequence or order in which patients become enlisted into the study. This number is to be entered on all subsequent study forms that are completed for each patient. It should be noted here that the number assigned at this time will later become a portion of a larger identification number to label and identify the blood specimens for each patient.

After a consent form is signed, a blood specimen is to be obtained before the patient undergoes surgery and/or transfusion. This specimen must be tested by the study center's laboratory for ALT level and HB_SAg.

If there is any doubt concerning a patient's continued eligibility, his willingness to participate, or his availability for follow-up, a study identification number should not be assigned.

Data reports to the coordinating center are not required for patients designated as "screen-only." For patients who are eligible for enlistment, data from the screening process are reported as part of the data acquisition program for enlistment. Screening information is reported on both Form A and Form 1. Form A is for convenience of the nurse; it does not have to be recorded or sent to the coordinating center.

Eligible patients must be identified prior to the event of reference and serum in an amount adequate for all study purposes must be collected. Whenever possible, serum samples should be taken several days prior to the event of reference to provide as extensive a baseline as possible. Effort should

be made to recover from the blood bank and from the laboratory residual sera obtained prior to the event of reference in order to increase the volume available for future studies. Patients identified after the event of reference are not eligible for the study.

TRANSFUSION - TRANSMITTED VIRUSES STUDY

FORM A - CRITERIA CHECKLIST

These criteria should be used when reviewing charts and interviewing patients. A "yes" answer to any question disqualifies the patient. Form 1 - Screening Interview and Enlistment Data, should be used and the data entered into the computer for an enlisted patient.

Location (address outside of study area).....

Malignancy, poor prognosis.....

Immunosuppressive drug.....

Bleeding disorder.....

Chronic liver disease.....

Continuing transfusion need.....

Other poor prognosis.....

Abnormal SGPT or SGOT recorded.....

Hepatitis anytime in life.....

Undiagnosed jaundice since age 15.....

Transfused within past 9 months.....

Exposed to viral hepatitis within past 6 months.....

Social exposure:

Household.....

Other.....

Occupational exposure:

As an Oral Surgeon.....

In a Hemodialysis or Oncology Unit.....

To human blood in a laboratory.....

In an Institution for Mentally Retarded.....

Other Increased risks.....specify.....

Other obvious disqualifying condition.....

.....

Comments.....

.....

Initials_____

Date_____

II-B. PROCEDURES FOR ENROLLMENT

CONFIRMATION OF ELIGIBILITY

For each enlisted patient, a re-evaluation period is initiated beginning with the date of the patient's surgery or first transfusion and lasting approximately two weeks. His medical condition is reviewed to determine if he still meets all of the study criteria. Enrollment is completed if all of the study requirements are fulfilled at the conclusion of the two-week period. Information concerning the outcome of enrollment is reported on Form 2 - Enlistment Outcome and Enrollment Data.

At the time of enrollment, a study patient must still meet all of the eligibility criteria stipulated during screening and enrollment.

It is important to realize, however, that eligibility by each criterion must be reconfirmed at enrollment because of the possibility of changes in the interval, i.e., if surgery reveals a medical condition associated with a poor prognosis, thus making complete follow-up uncertain, the patient is to be excluded.

As long as at least 1 of the 3 pre-enrollment ALT's (pre-day 7, day 14) is normal, then the patient is eligible for enrollment. The day 7 specimen is not mandatory for enrollment.

The patient must remain available and willing to participate in the follow-up phases of the study. It is better to exclude him from further consideration at this point than to have him refuse future blood collections.

All transfusions administered to a single patient must be given within a fourteen day period, beginning with the date of the first transfusion (i.e., the reference date).

In order to implicate donors as closely as possible, it is desirable that recipients transfused with as few units as possible be identified.

A patient who receives a massive number of units (sixteen or more) is excluded from enrollment.

Failure to obtain a sample of any donor unit transfused to a patient will be reason for exclusion also.

Three major tasks are accomplished during the re-evaluation period: 1) a reference date is established; 2) the patient's study status is identified; and 3) post-enlistment (7 days) and enrollment (14 days) specimens are collected.

1. The Reference Date

The reference date will subsequently be used to schedule all follow-up visits for a patient. Therefore, it is essential that this date be identified correctly. Several different sets of circumstances will determine a patient's reference date.

a) In the case of a patient who is transfused, the reference date corresponds to the date of the first transfusion.

b) For a patient who does not receive transfusion, the reference date is the date of surgery.

c) If no transfusion is received and no surgery is performed, the reference date is the date of enlistment (i.e., the date the consent form was signed).

2. Identification of Study Status

The patient who subsequently receives a transfusion of blood or unpooled derivative is identified as a "recipient." When a study patient is cross-matched for possible transfusions but does not receive any, he is identified as a "control."

Each investigative center will prepare and update a recipient control balance sheet. This will be done at regular intervals. It will be the responsibility of each center to balance cases. If controls are in excess of recipients, it is important that more recipients are enrolled; controls should be enlisted as ineligible until a balance is achieved.

A recipient may receive transfusions of his own blood (autologous units), blood or blood products of another donor (assuming that each donor can definitely be identified), or a combination of both. Once a recipient's study status is assigned, it is not subject to change. He may be transfused more than once, as long as the additional transfusions are administered within 14 days of the first transfusion (i.e., prior to enrollment).

If a control requires transfusion after enrollment, he is eligible to participate in the study as a recipient, assuming he is still eligible in terms of the study criteria. This patient should be re-enrolled with the same ID number, but a change in status. His reference date is then the date of transfusion and a new enrollment sheet should be completed.

3. Specimen Collection Requirements

A serum sample is to be collected 7 days after the reference date to establish any changes in ALT levels. A specimen must also be collected at day 14, enrollment.

A sample should be collected 7 days after the unit that is administered to a recipient.

The following procedures shall be carried out at each of the participating units on all specimens:

1) Patient's sera

   a) Alanine amino-transferase (ALT) should be tested each day using the Beckman TR System within 24 hours of sample collection if at all possible, and within 48 hours under any circumstances.

   b) HB_SAg by Ausria II-125 within 48 hours.

2) Donor's sera

   a) ALT should be done on sera (or plasma) within 24 hours of the time the unit is given to the recipient.

DATA FORMS AND REPORTING

Form 2

Form 2 - Enlistment Outcome and Enrollment Data - is used to record information from the phase of accession. The form is to be completed and the data computerized on all patients who are to be enrolled. All others will be considered "ineligible."

Halothane and like agents are to be listed as #1 in Type of Anesthesia. These "like" drugs are:

1) fluothane

2) pentothane

3) forane

4) enflurane

5) fluroxene.

If a patient receives a local anesthetic, this is classified as "none."

The questions on Form 2 correspond to the computer text used in the Enrollment Program of the Data Acquisition System created for this study.

If a patient is ineligible at this time, he is to be dropped from the study. Rarely will the reason for dropping a patient <u>not</u> be listed in the check-list. Number 11 should be checked <u>only</u> if the reason is not on the list.

TRANSFUSION DATA

Form 3

Form 3 - Transfusion Data - is used to identify all units of blood which were transfused into a recipient. The sequence in which the units are entered on the form determines the donor ID number. This form need not be completed for recipients who are ineligible at enrollment.

In the box in the upper right-hand corner give the institution code number, the recipient's study ID number assigned at the time of enlistment, and the recipient's name.

For each unit of blood, the following information should be given:

a) the date the blood was transfused
b) the type of material, using the codes indicated at the bottom of the form
c) name of the collection agency and the appropriate code indicated at the bottom of the form
d) collection agency's number for the unit of blood
e) hospital code number for the unit of blood
f) storage location of each corresponding specimen in the box that will eventually be shipped to NHLBI (The lab technician is responsible for assigning the space and entering the data on this form.)

DONOR IDENTIFICATION DATA

Form 4

Form 4 - Donor Identification Data - is completed for each donor identified on Form 3. These data are recorded at three different times:

Part A represents information at the hospital from the blood unit, chart, and/or blood bank.

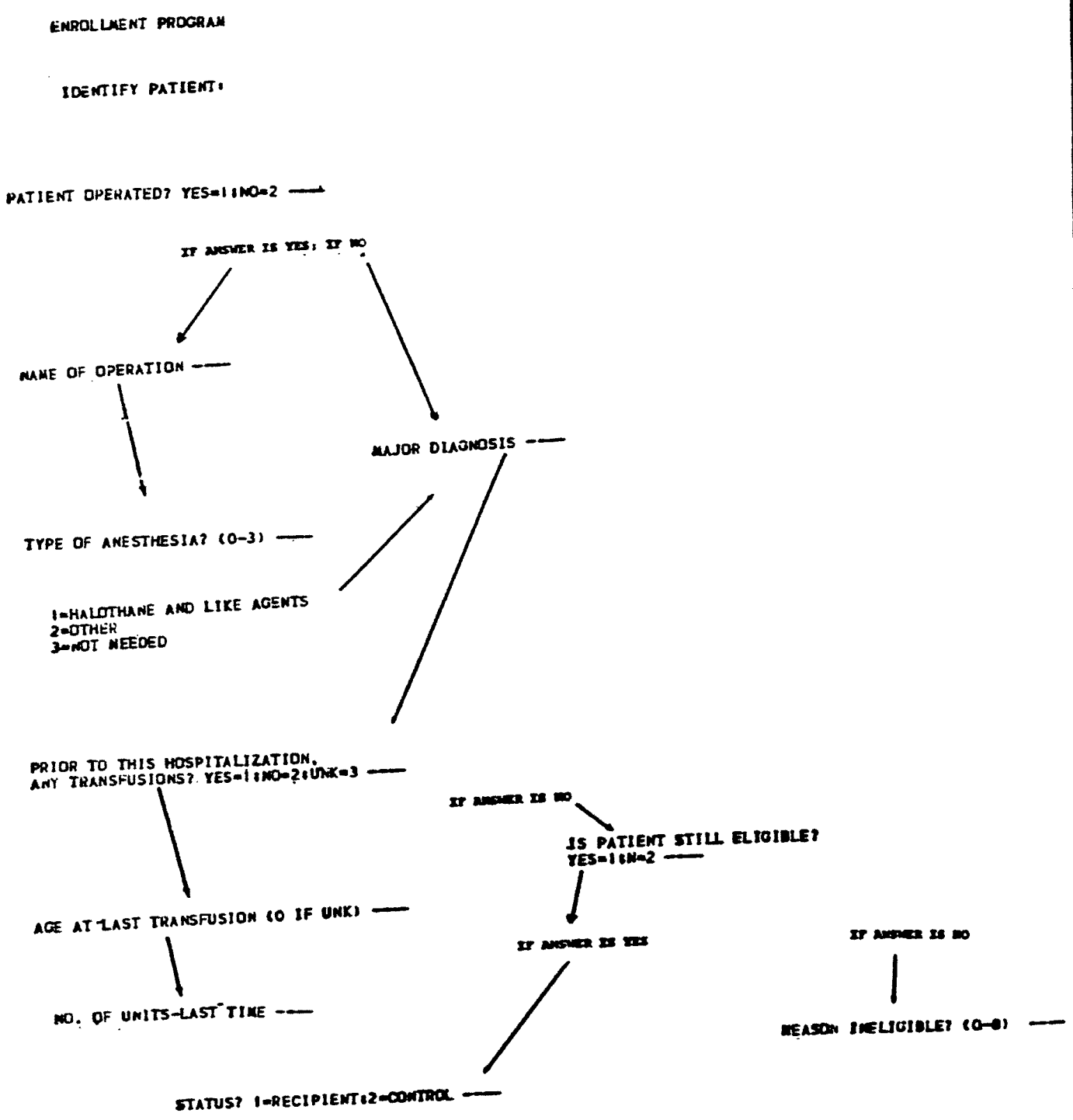
Part B is information available at the appropriate collection agency. Particular attention should be given to recording as much identifying information as possible in order to improve follow-up potential.

Part C is completed at the time donor follow-up takes place, in the event of implicated or control cases.

Collection agency acquired data (Part B) should be computerized using the Data Acquisition Program. Donor lab data also should go on this sheet. It is to be filled in under the space for comments, i.e.,

Comments: Date ( ) ALT ( )

ENROLLMENT INFORMATION
QUESTIONS PRINTED BY
COMPUTER
(ENROLLMENT PROGRAM 3)



ENROLLMENT INFORMATION
QUESTIONS PRINTED BY
COMPUTER
(ENROLLMENT PROGRAM 3)

TYPE OF RECIPIENT? (0-3) ___

1=OTHER BLOOD
2=UNN BLOOD ONLY
3=WASHED/FROZEN ONLY

NO. UNITS RECEIVED ___

MAJOR REASON? (0-4) ___

1=OPERATIVE LOSS
2=TRAUMATIC LOSS
3=GI BLEEDING
4=OTHER

REFERENCE DATE (YYMMDD):
RECIPIENT: DATE FIRST TRANSFUSION
CONTROL: DATE OPERATION
CONTROL: DATE ENTERED STUDY
DATE ___

ADDRESS ___

TELEPHONE NUMBER ___

BEST DAY ___

BEST TIME (AM/PM) ___

MAP CODE- HORIZONTAL ___

MAP CODE- VERTICAL ___

DATA CORRECT?

CORRECTION ___ ALPHA DATA:
GIVE PROBLEM NUMBERS
TO BE CORRECTED IN
RESPONSE TO
NEXT QUESTION ___
TO STOP CORRECTIONS
USE PROGRAM NUMBER
SHOWN

PROGRAM NUMBERS:
40=NAME OF PATIENT
41=CHART NO.
42=ZIPCODE
43=CROSSMATCH
44=NAME OF OPERATION
45=MAJOR DIAGNOSIS
46=REASON TRANSFUSED
47=REASON INELIGIBLE
48=ADDRESS
49=TELEPHONE NUMBER
20=STOP CORRECTIONS

PROGRAM NUMBERS:
52=PLACE SCREEN
53=REASON CROSSMATCH
54=SEX
55=AGE
56=RACE
57=DATE ENLISTED
58=PATIENT OPERATED
59=TYPE - ANESTHESIA
60=PRIOR TRANSFUSIONS
61=AGE LAST TRANSFUSION
62=# UNITS LAST TRANSFUSION
63=STATUS
64=TYPE OF RECIPIENT
65=# UNITS - RECIPIENT
66=REASON TRANSFUSED
67=REFERENCE DATE
68=REASON INELIGIBLE
20=STOP CORRECTIONS

```
PROGRAM (? FOR LIST)----?7

DONOR SEGMENT (1,2,3,4) ----?1


 DONOR DATA -- PART 1
 .
 .
 RECIPIENT ID:
ID NUMBER ----?5

 GREEN                  SARAH L.102030        90007
 AMPUTATION  ANEMIC        GANGRENE
CORRECT: Y=1;N=2 ----?1


 DONOR ID ----   ?201


 DATE TRANSFUSION (YYMMDD) ----  ?750103


 FORM OF TRANSFUSION:
 CODE (? FOR LIST) ----  ??


 FORM OF TRANSFUSION -- CODES:
 1 = WHOLE BLOOD
 2 = PACKED CELLS
 3 = PLATELETS
 4 = PLASMA
 5 = WHITE CELLS
 6 = OTHER

 FORM OF TRANSFUSION:
 CODE (? FOR LIST) ----  ?1


 COLLECTION AGENCY:
 CODE (? FOR LIST) ----  ?0


 COLLECTION AGENCY -- CODES:
 1 = RED CROSS
 2 = COMMUNITY BANK
 3 = HOSPITAL
 4 = CTS
 5 = CBP
 6 = OTHER

 COLLECTION AGENCY:
 CODE (? FOR LIST) ----  ?4
```

CHARACTERISTIC	ITEM #	PROG #	VALUE
PATIENT ID NUMBER -	5		
RECORD FOR SEGMENT - DONE			
-DONOR ID NUMBER			201.0
-DATE TRANSFUSION			750103.0
-FORM OF TRANSFUSION			1.0
-COLLECTION AGENCY			4.0

ADDITIONAL DONOR? Y;N ---- ?N

```
PROGRAM (Ø FOR LIST)----?7

DONOR SEGMENT (1,2,3,4) ----?2


 DONOR DATA -- PART 2
 (FROM COLLECTION AGENCY)

 RECIPIENT ID:
ID NUMBER ----?5

 GREEN                  SARAH L.1Ø2Ø3Ø      9ØØØ7
 AMPUTATION  ANEMIC        GANGRENE
CORRECT: Y=1;N=2 ----?1


  DONOR ID ----   ?2Ø1


  SEX: 1=M;2=F ----   ?1


  RACE: 1=OTHER;2=BLACK   ?2


  AGE:
  1=AS DATE OF BIRTH
  2=IN YEARS
  WHICH ----  ?1


  DATE OF BIRTH (YYMMDD) ---- ?32Ø225


  CHARACTER OF DONATION:
  1=VOLUNTARY
  2=REPLACEMENT
  3=PAID
  CODE ----   ?3


  NUMBER PRIOR DONATIONS (99=UNK)---- ?2
```

CHARACTERISTIC	ITEM #	PROG #	VALUE
PATIENT ID NUMBER -		5	
RECORD FOR SEGMENT - DTWO			
-DONOR ID NUMBER			201.0
-SEX OF DONOR			1.0
-RACE			2.0
-AGE IN YEARS			43.8
-CHARACTER OF DONATION			3.0
-NUMBER PRIOR DONATIONS			2.0

ADDITIONAL DONORS? Y;N ---- ?N

II-C. ROUTINE FOLLOW-UP

The first follow-up interview is to be scheduled four weeks after the reference date. Subsequent visits are to be planned at 6, 8, 10, 12, 15, 18, 21, 24, and 40 weeks after the reference date.

A blood specimen of 30 ml is to be collected at each visit. An interview is given at the time the blood is drawn to determine the health status of the patient and its association or lack of association with hepatitis.

At each of these routine visits, a high index of suspicion for hepatitis should be maintained. If a patient has any complaints, the interviewer should attempt to determine if they relate to the condition that caused the patient's hospitalization or may be indicative of incipient viral hepatitis.

In the event the patient's symptoms are suggestive of a diagnosis of hepatitis, special follow-up of the patient is to be initiated.

Form 5

Form 5 - Routine Patient Follow-up Data - is to be completed for each visit.

If the patient's symptoms or complaints suggest a possible diagnosis of hepatitis, complete the Special Follow-up Form.

TERMINATION OF ROUTINE FOLLOW-UP - INELIGIBLE/DROPPED

All reasonable effort is to be made to keep patients under follow-up observation until the end of 40 weeks. In spite of these efforts, it is recognized that a certain percentage of patients will refuse to continue participation and/or become impossible to locate. Additional problems or situations involving the patient's social, work, and family life also may result in termination of follow-up.

Several disqualifying conditions are:

1) If a member of the patient's household develops an overt case of hepatitis.

2) Any sexual contact with a known case or carrier.

3) Being started on isoniazid.

QUESTIONS PRINTED BY COMPUTER
(ROUTINE FOLLOW-UP PROGRAM 4)

ROUTINE FOLLOW-UP

IDENTIFY PATIENT:

DATE OF INTERVIEW (YYMMDD) ——

PLACE OF INTERVIEW (0-5) ——

1=STUDY CENTER
2=HOME
3=TELEPHONE
4=BUSINESS
5=OTHER

IF THE ANSWER IS CODE 3, the following will be printed.

FOR TELEPHONE INTERVIEWS:
CONFIRM THAT DATE OF INTERVIEW AND DATE OF LAB REPORT ARE THE SAME BEFORE ENTERING LAB DATA.

SINCE LAST FOLLOW-UP REPORT:

-PATIENT OPERATED? YES=1;NO=2 ——

-PATIENT TRANSFUSED? YES=1;NO=2 ——

-EXPOSED TO HEPATITIS? YES=1;NO=2 ——

-TAKING ISONIAZID? YES=1;NO=2 ——

-TAKING METHYLDOPA? YES=1;NO=2 ——

-HEALTH STATUS (0-3) ——

1=INDICATIONS-POSSIBLE HEPATITIS
2=SYMPTOMS-NOT HEPATITIS
3=ENTIRELY WELL

FOLLOW-UP PLANS (0-5) ——

1=AS PROTOCOL SPECIFIES
2=INELIGIBLE FOR CONTINUING FOLLOW-UP
3=UNWILLING
5=COMPLETED

DATA CORRECT?

CORRECTION OF DATA RECORD:
THE SPECIAL TESTING INVOLVED PRECLUDES SIMPLE CORRECTION OF DATA ENTRIES. ONCE THE FIRST PROGRAM NUMBER IS GIVEN, ALL SUBSEQUENT ANSWERS MUST BE RE-ENTERED.

PROGRAM NUMBERS FOR CORRECTIONS:
5=DATE INTERVIEW
6=PLACE
10=PATIENT OPERATED
11=TRANSFUSED
12=EXPOSED TO HEPATITIS
13=ISONIAZID
14=METHYLDOPA
15=HEALTH STATUS
19=SINGLE INDICATOR-(?)HEPATITIS
35=FOLLOW-UP PLANS

FOR PATIENT TRANSFUSED

RECIPIENT

CONTROL

PATIENT NO LONGER ELIGIBLE FOR CONTINUING FOLLOW-UP. SO INDICATE IN NEXT QUESTION.

PATIENT IS POTENTIALLY ELIGIBLE TO RE-BEGIN STUDY PROCESS.

THE PRESENT FOLLOW-UP PROCESS SHOULD BE CONTINUED INDICATE IN ANSWER TO NEXT QUESTION - CODE 1

FOLLOW-UP PLANS (0-5)——

1= AS PROTOCOL SPECIFIES
2= INELIGIBLE FOR CONTINUING FOLLOW-UP
3= UNWILLING
4= NOT AVAILABLE
5= COMPLETED

II-D. SPECIAL PATIENT FOLLOW-UP

PROCEDURES

Special follow-up is to be initiated on the basis of either of the following:

1) An abnormal laboratory test on a specimen obtained during routine follow-up.

2) Symptoms suggestive of hepatitis or observation of jaundice at the time of a routine follow-up visit.

Visits are to be scheduled at weekly intervals and a blood specimen is to be obtained at the time of each visit. Weekly follow-up is continued until hepatitis is ruled out or established as a definite case by the principal investigator at that center. Once the criteria for hepatitis have been met, the special follow-up should be terminated and patient follow-up should return to the original routine schedule. All patients, including those diagnosed as having hepatitis, are followed for 40 weeks.

Criteria used for abnormal laboratory results are as follows:

a) An ALT level greater than 44 IU.

b) A positive test result for hepatitis B surface antigen.

If hepatitis is suspected because of laboratory tests, the first special follow-up visit is to be scheduled in the week following the routine follow-up visit at which the abnormal specimen was obtained.

If hepatitis is suspected on the basis of symptoms disclosed at the time of the routine follow-up visit, then a special follow-up interview should take place at the same time.

If 3 specimens, collected over 14 days, have ALT values greater than 44 IU but less than 90 IU, then the special follow-up is to be terminated and the patient returned to the routine follow-up schedule.

II-E. DONOR FOLLOW-UP

Perhaps one of the most important aspects of the study is the donor follow-up. Compiling information, comparing lab results and special follow-up should provide the study with invaluable information.

Most important is getting the necessary information regarding the donor from the various collection agencies. It is necessary to get as much information as possible from the agencies. Not all donors will be sought, only those who will be used as controls for the implicated donors and the implicated donors themselves.

Donors are classified as implicated at the time the recipient meets the criteria for transfusion-associated hepatitis. Controls will be chosen from a group of donors at the same collection agency around the same time, according to the rules listed on page II-30.

These controls are to be treated the same as the implicated. The interviewer is not to tell the donor if he is a control or implicated. Therefore, it will be mandatory to keep the interviewer blinded. If, for some reason, the interviewer is unblinded, the fact should be noted in red in the upper right hand corner and mailed to the coordinating center. It is <u>not</u> to be entered by the individual center.

If a donor cannot be located, Form 4, Section 3 - Donor Follow-up Status - is to be completed. Copies should be sent to the coordinating center.

For the initial contact with the donors, letters will be sent out advising them of the study and asking for their cooperation. This will be followed by a phone call to make an appointment.

The Red Cross, when notified by the study group, will mark their files as such; they will not contact the donor until the center has time to interview and get a serum sample. The National Red Cross uses, for permanent deferment, the following criteria:

1) If 1 person receives 2 units and contracts hepatitis, both donors are permanently deferred.

2) If the donor has any history of hepatitis or being HB_sAg positive.

Rules for Matching an Uninfected Recipient to an Infected Recipient for Special Follow-up of Control Donors

(1) The matching is done as soon as the diagnosis of viral hepatitis is reasonably certain in the recipient (hopefully, within 2 to 4 weeks at most from first abnormalities in the person suspected of hepatitis).

(2) Match only for infected recipients who received five or fewer units, unless HBsAg test shows conversion from negative to positive. In latter instance, match uninfected recipient of one to 16 units.

(3) One wishes to avoid having to rematch an infected recipient with a second uninfected recipient because the <u>first</u> matched patient subsequently evidenced infection. For this purpose, the uninfected recipient chosen should be one that has been in follow-up longer. The project coordinator, therefore, should scan ID's in <u>descending</u> numerical order.

(4) To identify unimplicated donors for special follow-up, choose the first uninfected recipient who recieved the same number of units as the infected recipient.

(5) If after review of the 50 preceding patients there is no uninfected recipient who can be matched to the infected recipient on the basis of the same number of donor exposures, choose the 20th, 21st, etc., until the number of unimplicated donors is equal to or greater than the implicated donors to the infected patient.

Follow-up will be initiated on non-A, non-B and HBV as follows:

1) Non-A, non-B: This group will be divided into two categories: a) those receiving 5 or less units; and b) those receiving 6 or more. The 5 or less donors all are to be contacted, interviewed, and a blood specimen obtained. If there are 6 or more donors, follow-up is optional.

2) Hepatitis B: All donors must be interviewed and bled, regardless of the number.

II-F. SPECIAL DONOR FOLLOW-UP

This will probably give us the most valuable information from the study. Therefore, this area should receive special attention. The interviewer, as stated previously, will be blinded. This will serve a twofold purpose: first, he will treat each donor in an equitable manner with no differences in attitude; secondly, there will be no temptation to reveal to the donor his implication in a hepatitis case.

It will be a one time only follow-up. They will be asked the questions on Form 7 - Special Donor Follow-up, given a consent form to sign, and two red-topped tubes (30 cc) of blood will be drawn.

They should be asked if they would like the results sent to their doctor and, if so, a name and address should be obtained.

SPECIAL DONOR FOLLOW-UP DATA

Form 7

Part A - Patient Identification

1-8 This will already be given to the phlebotomist when he or she prepares to contact the donor.

Part B - Additional History

1-4 Self-explanatory

5 Self-explanatory

6 Marital Status. Do not assume that "never married" and "single" are the same. If they say they are single, make sure that means that they have never been married.

7 Number of people in household - people actually living there and staying overnight, whether or not related (excluding the donor).

8 Usual Occupation - put in as best you can the category in which one is generally employed.

9 Actual occupation at the present time.

10 Self-explanatory

11 Self-explanatory

12 Reason for donation. If commercial blood bank, they are paid. If hospital or Red Cross they could either be volunteers or replacement.

13 Estimated number of donations in lifetime. If not sure, try to estimate to the closest figure. Only write down 0 if the reference donation was the first in his lifetime.

14 Plasmapheresis donor at any time. Explain that this could be anything outside of whole blood, as they might not quite understand what this is. Many people who sell their blood to blood banks also sell it to plasma centers as this can be done much more frequently.

15 Transfused at any time in life. This is very important to the study; if they are not sure but have been operated on, etc., find out what was done or the circumstances surrounding why they think they might have been. This can then be given back to someone at the

center who will be better able to determine whether or not blood could have been given. If they are not sure of the age, approximate the time and the same supplies to the number of units.

16 Hepatitis at any time in life--Most people here will say no, as that is asked when they go to give blood.

17 Jaundice at any time in life--if the answer here is yes, find out the age, if they were under a doctor's care and what the circumstances were surrounding the jaundice.

18 Work Exposure--this means working in a situation where you are exposed to the type of people who could make you more likely to be exposed. It does not mean you once worked with someone who came down with hepatitis. If the answer to this is yes, find out the type.

19 Tattoo at any time in life--self-explanatory.

20 Personal contact with individual during acute stage of hepatitis. Someone at work is much different than someone living in the same house or even a sexual partner and this should all be recorded.

Any household member transfused any time in life--this refers only to while they were living in the same household as the donor. If the answer to this is yes, find out what household member. It is quite different if it is a husband or wife than if it is a brother.

Any household member have hepatitis any time in life--this means while living in the same household with the donor--it does not mean that the wife had hepatitis when she was 10 years old. Also find out who the household member was and write that in.

Any household member exposed to hepatitis in the last two years--this could be any type of exposure at all. A child could have been exposed at school, or someone at work. Again, write in the member that had the exposure.

EXAMPLES OF EMPLOYEES IN THE VARIOUS CENSUS BUREAU CLASSIFICATIONS[o]

PROFESSIONAL-TECHNICAL

Accountant
Actor
Pilot
Artist
Chemist
Clergyman
Physician
Draftsman
Engineer
Lawyer
Musician
Nurse
Teacher
Technician

CRAFTSMEN-FOREMEN

Carpenter
Electrician
Machinist
Mechanic
Plasterer
Plumber
Metal Worker
Foreman
Brickmason
Boilermaker

CLERICAL

Bookeeper
Clerk
Telephone Operator
Stenographer
Secretary
Typist
Mail Carrier
Cashier
Collector
Office Machine Operator

FARMERS

Farm Owner
Farm Tenant
Farm Manager

OPERATIVES

Bus Driver
Seaman
Taxicab Driver
Meat Cutter
Deliveryman
Chauffeur
Apprentice
Switchman
Stationary Fireman
Machine Operator
Mine Operative
Laborer

SALES

Salesman
Sales Clerk
Advertising Agent
Demonstrator
Insurance Agent
Real Estate Agent

SERVICE WORKERS

Hospital Attendant
Barber
Bartender
Housekeeper
Cook
Elevator Operator
Fireman
Janitor
Policeman
Waiter
Waitress
Porter
Fountain Worker

MANAGERS-OFFICIAL PROPRIETORS

Buyers-Purchasing Agent
Department Heads
Inspector
Superintendent
Postmaster
Union Official

LABORERS

Common Labor
Fisherman
Gardener
Lumberman
Longshoreman
Car Washer
Car Greaser

FARM LABORERS

Farm Laborer
Farm Wage Worker

[o]"Statistical Abstract of the United States," Bureau of the Census, U.S. Department of Commerce (1958 Edition).

*Korzekwa, Fred F., Jordon, W. Quinn, and Alsever, John B. The Blood Donor: I. Who are our Blood Donors? An Analysis of Social and Other Characteristics of 12,759 Donors. Amer. J. Med. Sci., pp 70/36-46, July 1960.

CONSENT FORM TO PARTICIPATE

IN A STUDY OF TRANSFUSION-TRANSMITTED VIRUSES

I, _____, freely agree to participate in a study undertaken by the _____ in cooperation with the United States Public Health Service. The purpose of the investigation is to assess the risk of transmitting viruses by blood transfusions of hospitalized patients. At least one aspect of this problem may be that available tests are not sufficiently sensitive or rapid to exclude the administration of units of blood which contain viruses. This study is expected to be able to speed the application of any new tests which may be developed.

I agree to allow 30 ml of blood to be taken from an arm vein at the present time. I am willing for this specimen, and a sample of my blood administered to a hospital patient also participating in this study, to be tested for evidence of hepatitis infection by presently available methods and by other methods that may be developed in the future. I also agree to its future testing for any purposes that may make blood transfusion safer. I have, however, the right to stop or limit participation at any time, and to withdraw consent for any or all further testing of blood specimens already taken.

I recognize the only benefit to me may be the probable benefit of the study to the many persons (possibly including myself) who will need blood in the future. I am told that the only hazard to me of participating will be the very small risk of a reaction to inserting a needle in my vein.

I agree that personal and medical information (sex, age, diagnosis, etc.,) may be copied from my record at the blood bank. I have been assured that this information and the test result will be kept confidential, and will be utilized only for analyses in connection with this investigation. Reports of these analyses will be published in appropriate professional journals.

I have been given an opportunity to ask questions regarding my participation in this study and have discussed it with the investigators to my satisfaction. The investigators agree that they will provide any further information that I desire in the future.

My consent to participate is freely given.

_____ _____

Investigator's signature Donor's signature

Date_____ Date_____

II-G. CASE EVALUATION

EPISODE

An episode is an occurrence or a series of occurrences suspected of being related to infection by the virus causing hepatitis.

The events initiating an episode are:

1) The occurrence of symptoms compatible with or suggestive of viral hepatitis

2) The occurrence of asymptomatic clinical jaundice

3) The occurrence of a serum alanine aminotransferase level ≥45 IU

4) The appearance of HB_SAg positivity

5) Seroconversion to anti-HB_S, anti-HB_C, or anti-HA positivity.

DESCRIPTIVE COMPONENTS FOR CATEGORIZATION OF AN EPISODE

1) The etiologic agent causing the episode

2) The certainty with which that agent can be assigned etiologic responsibility

3) The occurrence or absence of evidence for hepatitis (i.e., necrosis and inflammation of the liver)

4) The adequacy of the evidence for a diagnosis of hepatitis

5) If diagnosed as hepatitis, the severity of the case

6) The time relationship of the episode to the reference date

7) The evidence for persistence or resolution of infection and/or hepatitis.

8) The duration of an episode shall be that period between the first and last abnormal ALT values (≥45 IU).

An episode associated with suspected hepatitis will be labeled with the following components as part of the final evaluation:

1) An operational definition - hepatitis or hepatitis virus infection

2) A clinical definition - symptomatic or asymptomatic

3) A definition of derangement in bilirubin metabolism, icteric, hyperbilirubinemic or non-mobilirubinemic disease

4) An etiologic definition - hepatitis B virus, etc., or no etiology identified.

The following is a list of definitions used to fulfill the practical needs of the study:

Hepatitis will be applied to episodes in which there is evidence of hepatic disturbance (presumable necrosis) sufficient to elevate the serum alanine aminotransferase activity to a level twice the upper limit of normal on one occasion (>90 IU) and, on a second occasion, ≥45 IU 3 to 17 days *before or after* the time of the previous abnormality. Lesser degrees of abnormality shall not be considered hepatitis even if accompanied by serologic evidence of hepatitis virus infection.

Hepatitis Virus Infection will be applied to episodes in which there is serologic evidence for that occurrence but the criteria for hepatitis are not met.

Symptomatic will be applied to episodes in which the patient has at least 2 symptoms on 2 visits or 3 symptoms on 1 visit.

Asymptomatic will be applied to episodes of hepatitis in which criteria for symptomatic disease are not met. The term should not be used in instances of hepatitis virus infection. If symptoms compatible with viral hepatitis occur in the absence of aminotransferase activity, the episode should be considered non-hepatic.

Icteric will be applied to episodes where the bilirubin is >2.0 mg or overt jaundice is seen by a trained observer.

Hepatitis B Virus will be identified as the responsible etiologic agent if:

a) HB_sAg appears in the serum during follow-up. If present in the first post-reference serum (day 7 or day 14), at least one measurement subsequently shall be significantly bigger to rule out passive antigenemia in transfused patients.

b) Anti-HB_s appears in the serum for the first time or increases in titer by $\geq$ fourfold in a specimen taken 28 days or more after the last transfusion.

III. SPECIMENS

III-A. LABELING OF SPECIMENS

Each patient (whether recipient or control) is assigned an ID number for the Study. This consists of two components or segments separated by a hyphen. Specimens related to an individual patient are then specifically identified by a third segment, linked to the other two by a hyphen. Thus, the specimen label will carry a total of three segments.

FIRST SEGMENT: Institutional identification

1 = Washington University School of Medicine

2 = University of California, Los Angeles

3 = Baylor College of Medicine

4 = New York Blood Center

SECOND SEGMENT: Patient identification

This consists of a 4-digit number from 0001 through 9999 at each institution. Leading zeros (e.g., 0003) should be retained on all specimen labels to minimize errors in their handling. This ID is assigned by the computer at the time of enlistment. Not all patients enlisted will be enrolled, so that ID's of the patients to be actually followed will not be a closed set of numbers. This was not intended in design of the system, but there proved to be no other practical way in the computer program of appropriately connecting the enlistment data to subsequent components.

Notice that the ID does not differentiate between transfusion recipients and hospitalized controls. This is because at the time of enlistment it is not and should not be possible to predict the final category for the patient (a designation applicable to both recipients and controls).

The patient identification is also applied to donor(s) of any unit(s) the patient may receive. This is done in order to link easily donors to the appropriate recipient. Differentiation between patients and recipients is made in the third segment of the ID, as described below.

THIRD SEGMENT: Specimen identification

a. The first digit of the third segment differentiates between a patient specimen and a donor specimen.

Patient = 1

Donor = 2

b. The final two digits of the third segment represent either:

(i) If the first digit is a "1", then the last two digits are the number of the particular specimen in the sequence of sera collected for the patient. The sequence begins with "00" for the specimen collected prior to the reference date. The reference date will usually be the date of first transfusion (recipient) or the date of operation (control). If more than one specimen is obtained prior to the reference date, then the first is designated by a decimal, followed by "1", and subsequent specimens in sequence by ".2", ".3", etc.

If the patient is enlisted because of cross-matching for GI bleeding and becomes a control (i.e., is not transfused), then his reference date becomes the day of enlistment. The specimen taken at enlistment then becomes the "100" serum even though it is coincident with, rather than before, the reference date.

(1)00.1 = (patient) first specimen taken prior to reference date, if more than one specimen is obtained in the pre-reference period.

(1)00.2 = (patient) second specimen taken prior to reference date.

(1)01 = (patient) first specimen after the reference date, ordinarily at week 1 (7 days).

(1)02 = (patient) second specimen after the reference date, ordinarily at week 2 (14 days).

(1)03 = (patient) third specimen after reference date, ordinarily taken at week 4 (28 days).

Notice that the last two (pre-decimal) digits refer to the specimen sequence and not to the week of follow-up (i.e., the serum obtained during the 4th week is "103", not "104". This avoids the complications of an additional decimal system if the frequency of serum sampling increases because of special follow-up visits for suspected hepatitis.

If a follow-up visit is missed, the next specimen is nonetheless labelled with the next number in the sequence. Thus, the post-reference date sera will always be a closed set.

If the patient is enrolled as a control and becomes a recipient because of transfusion during follow-up, he is not assigned a new ID number. The previously acquired specimens should be left in the box and compartments in which they have already been stored, without relabelling. The new specimens will continue in the same sequence (e.g., ...108, 109, etc.) and should be stored in the same box and compartments as before. The entry of the new reference date will revise the time sequence accordingly on the computer display, a copy of which will be on file at the repository. An appropriate notation should be made, however, on the PATIENT SPECIMEN LOG (see below).

(ii) If the first digit of the third segment of the ID is a "2", then the last two digits become a donor ID. The assignment of such a donor ID is accomplished when the fact of transfusion of his blood is entered onto Form 3-TRANSFUSION DATA. An example of Form 3 follows.

O.M.B. No. 68-876018
Approval Expires December 1980

FORM 3 - TRANSFUSION DATA

TRANSFUSION-TRANSMITTED VIRUSES STUDY

Institution _____

Patient ID No. _____

Name _____

DO NOT LIST AUTOLOGOUS UNITS ON THIS SHEET

Donor ID No.	Date Transfused (YYMMDD)	Form of Transfusion (*use codes)	Collection Agency (**Code)	Collection Agency Name (if other)	Collection Agency No.	Hospital code Number	Specimen Storage		
							Box	Comp	P
201									
202									
203									
204									
205									
206									
207									
208									
209									
210									
211									
212									
213									
214									
215									

*1 = whole blood
2 = packed cells
3 = platelets
4 = plasma
5 = white cell
6 = washed/frozen cells
7 = other (specify) _____

**1 = Red Cross
2 = Community
3 = Hospital
4 = CTS
5 = CBP
6 = Doctors'
7 = other (specify) _____

Form 3

Observe that in the left-hand column the donor identification is provided in the format of the third segment of the specimen label.

For most donors there will be only one specimen. For implicated donors and control donors who enter special donor follow-up, however, there will be an additional sample(s). The original sample from an implicated donor becomes one of the most valuable specimens in the entire study, and a very special procedure in their handling is justified.

For implicated donors, the original specimen (i.e., all aliquots of the serum or plasma obtained from the "pilot" tube or residual blood in the donor bag) should be relabelled if that donor box has not been shipped to the NIH repository. The relabelling should be in red with the same donor number, to which .1I is added to give a final designation as X-XXXX-2XX.1I. This indicates that it was the first specimen obtained from the particular implicated donor.

The serum sample collected from the implicated donor at the time of the special donor follow-up (i.e., at the time he is implicated) should be labelled in red according to the format X-XXXX-2XX.2I. The aliquots from follow-up will be placed in the next available position in the currently used donor box. Thus, the initial and follow-up implicated donor specimens are likely to be in different boxes (making imperative the careful recording of location on the PATIENT SPECIMEN LOG (see Section VII).

For the samples from control donors at the time of special donor follow-up, relabelling of the original specimen (i.e., the serum or plasma derived from the pilot tube or donor bag) from X-XXXX-2XX to X-XXXX-2XX.1C would be desirable to show that another (later) specimen exists from the same donor, but will probably not be feasible because boxes are likely to have been transferred to NIH by the time the control is designated as such.

The second specimen from the control donor, taken at the time of special donor follow-up, should be labelled according to the format X-XXXX-2XX.2C. It will be locatable by an appropriate entry on the PATIENT SPECIMEN LOG (see Figure 6).

CHARACTER OF THE SPECIMEN: (Serum or Plasma)

The specimens may be either serum or plasma. For some tests to be done at future times, plasma may not be suitable and defibrination will be required before use. If a sample is not labelled as to its character, therefore, erroneous results may be unknowingly obtained, or material wasted even if the fact of error is discovered. Each specimen, therefore, should be labelled "Pl" or "Se" in the lower right-hand corner of the label.

If all (i.e., each and every) specimen is serum or plasma, then the box should be labelled on the the top "All serum" or "All Plasma."

III-B. STORAGE OF SPECIMENS FOR NHLBI

The agreement with NHLBI stipulates that 5.0 ml of each serum collected from a patient, whether recipient or control, and 5.0 ml of each specimen (if more than one) from each donor, is to be set aside for shipment to and permanent storage at NHLBI. The creation of this bank represents the single most important function of the TTV Study, so that priority should be given to this aspect at all times.

Other understandings concerning the specimens reserved for NHLBI include the following:

1. The specimen may be either serum or plasma. Each has some possible advantage in relation to future tests, and it is impossible to anticipate which will be preferable for procedures not yet devised. It is most practical, therefore, to leave the reserve specimen in the form in which it is obtained.

2. Specimens may be collected in non-sterile vacuum tubes. In handling, however, every effort should be made to minimize contamination, with use of sterile pipettes for transfer and sterilized vials for storage. Storage should be in a low-temperature freezer (-50° to -70°) at all times.

3. The specimen will be stored as two aliquots of 2.5 ml each.

4. No preservative to prevent or minimize microbial growth should be used because of its potential effect upon recovery of agents if techniques for cell culture of transfusion-transmitted viruses are applied at some future time.

5. Adequacy of the specimen with respect to the 5.0 ml volume will be routinely monitored by the person responsible for receiving the specimens at the NIH repository. This is Mr. Rodney Miller, Flow Laboratories, Rockville, MD, (301) 881-2900.

6. The boxes to be used for storage should be 13 1/8" x 9" x 2 3/8" in outside dimensions, each with 24 compartments of equal size. Those manufactured by Vlcheck (Composite box S-M-824) meet this stipulation and should be used unless otherwise agreed with the coordinating center and the NHLBI Project Officer.

7. Each box should be labelled on the top as follows:

TRANSFUSION-TRANSMITTED VIRUSES
NHLI CONTRACT SPECIMENS

PATIENT BOX ___-___

SPECIAL BOX ___-___

DONOR BOX ___-___

CHARACTER OF SPECIMENS

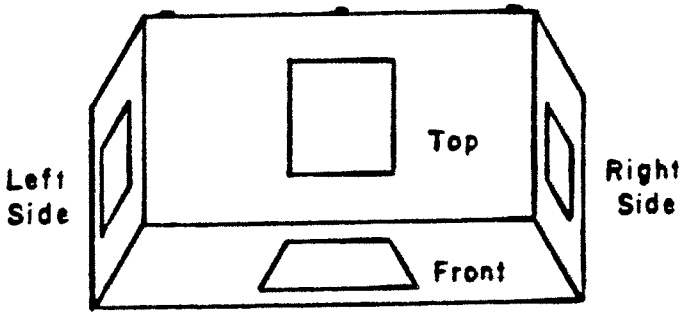
ALL SERUM ___

ALL PLASMA ___

AS LABELLED ___

The label should be completed to show the institution's number before the hyphen, and the box number after the hyphen.

Each box should also be labelled on both ends and the front side (i.e., the long-dimension side with the hinge away from the observer, as illustrated in the following diagram).

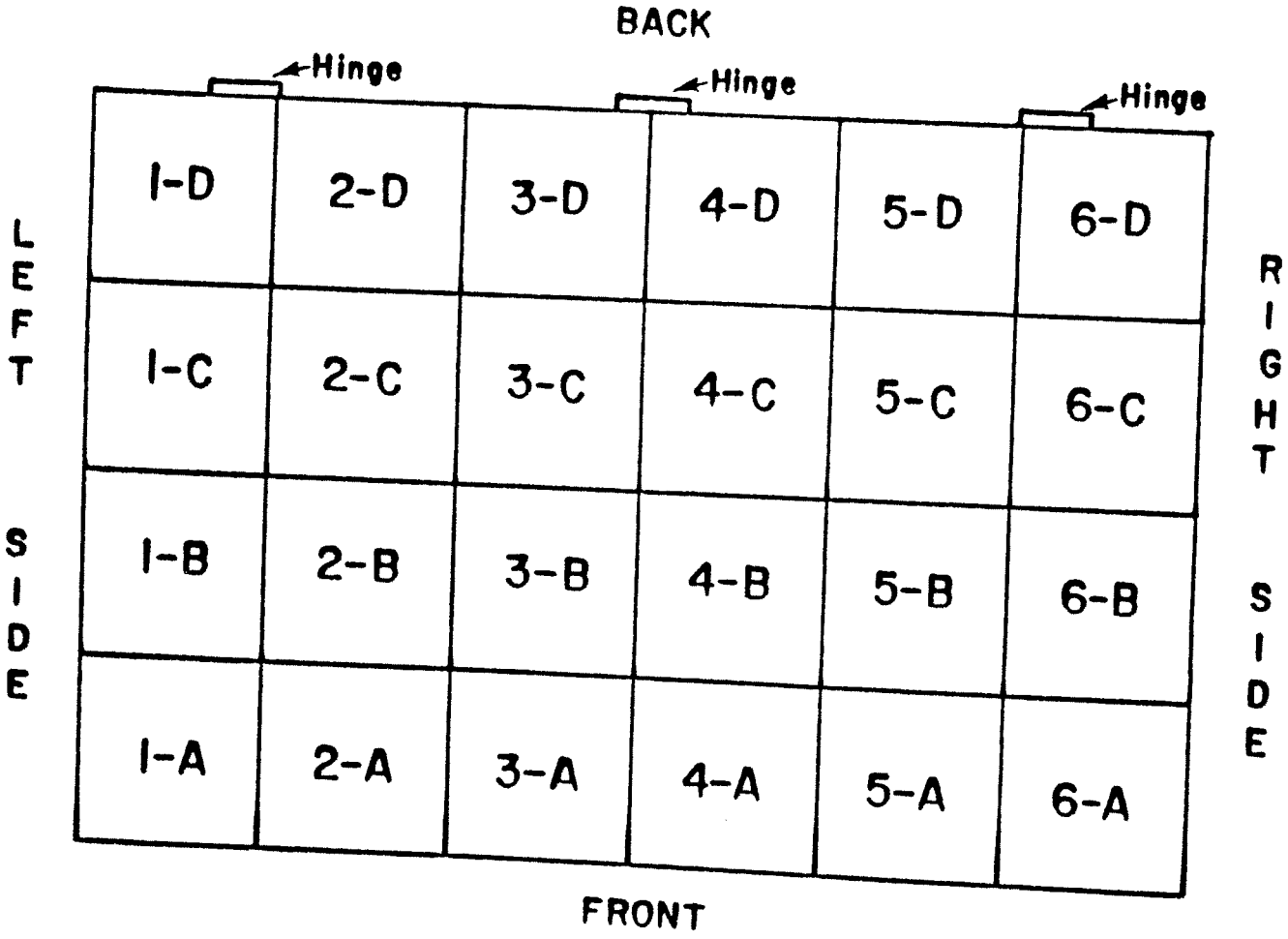


8. There will be three categories of boxes: (1) PATIENT BOXES, which will hold consecutively numbered specimens from patients whether they are recipients or controls; (2) SPECIAL BOXES, which will hold patient specimens when they exceed the capacity of allocated space in Patient Boxes; (3) DONOR BOXES, which will hold donor specimens in the sequence in which they are collected.

9. Each PATIENT BOX is intended to hold specimens from six patients. In each box, four compartments will be reserved for each patient. These begin at the front (the side opposite the hinge) and proceed to the back, occupying the compartments designated as "A" through "D", as illustrated in Figure 1.

Figure 1

Description of Box for Storage of NIH Specimens
Transfusion-transmitted Viruses Study



10. Each compartment will hold nine vials of one fluid dram (3.7 ml) each. Within each compartment, therefore, there are nine positions which are numbered from right to left, row by row (FIGURE 2) The two vials of the first specimen for a patient (e.g., 1-0038-100) should be placed in the forward compartment (1A) beginning in the forward, left-hand corner (position 1 and 2). One aliquot of the next specimen for that patient (1-0038-101) should go to the immediate right (position 3) of specimen 1-0038-100 in the forward row of the comparment, the other aliquot going to the left-hand side in the second row (position 4). This sequence is illustrated in FIGURE 3.

11. The use of four comparments allows the storage of two 2.5 ml aliquots from 18 specimens. Each patient uneventfully completing follow-up will have a minimum of 13 specimens. Thus, space is provided for four to six special follow-up specimens.

12. The SPECIAL BOX will also be used for patient specimens. but only after the allocated space in the PATIENT BOX is exhausted. Compartments in the SPECIAL BOXES should be filled in the sequence 1-A, 1-B, 1-C, 1-D, 2-A, 2-B, ...6-D. The positions are filled from left to right, row by row. For example, at institutuion #1, patient 1-0002, a control, and 1-0005, a recipient, develop hepatitis. 1-0002 is followed twice a week beginning at the 16th week. Abnormalities persist for three additional weeks and then subside, so that the patient reverts to routine follow-up at the 21st week. Thus, two aliquots each of 20 specimens are to be stored.

7/ 9/74	pre-surgery	1-0002-100
7/18/74	week 1	1-0002-101
7/25/74	2	1-0002-102
8/ 8/74	4	1-0002-103
8/21/74	6	1-0002-104
9/ 2/74	8	1-0002-105
9/19/74	10	1-0002-106
10/ 3/74	12	1-0002-107
10/25/74	15	1-0002-108
10/28/74	16	1-0002-109
10/31/74	16	1-0002-110
11/ 4/74	17	1-0002-111
11/ 7/74	18	1-0002-112

Figure 2

Position Numbers (see item #10, III-10)

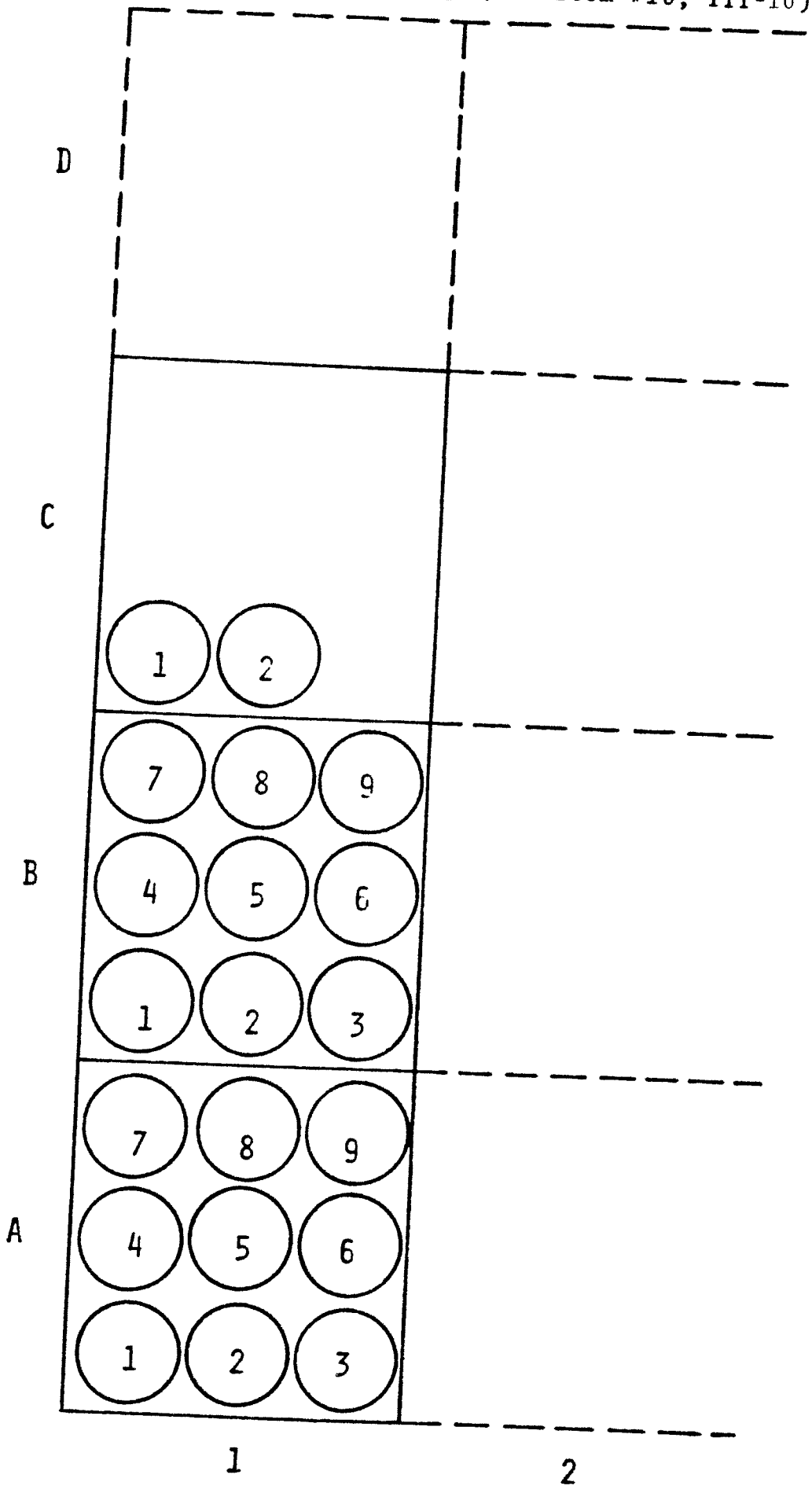
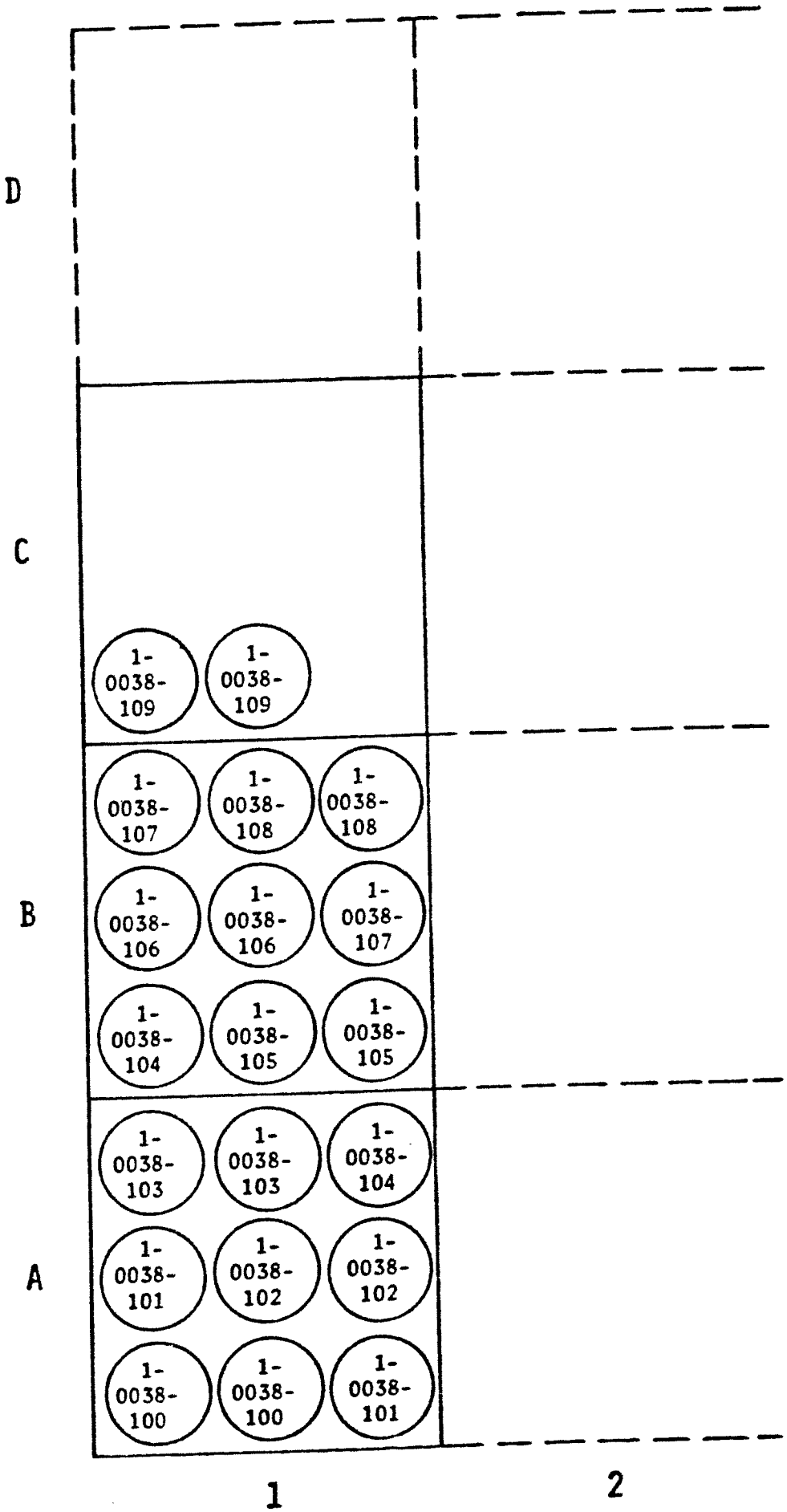


Figure 3

Patient Box (see item #10, III-10)



11/18/74	18	1-0002-113
11/21/74	18	1-0002-114
11/25/74	19	1-0002-115
11/28/74	19	1-0002-116
12/12/74	21	1-0002-117
1/ 3/75	24	1-0002-118
4/15/75	40	1-0002-119

Patient 1-0005 has his first abnormality during the 20th week and is followed weekly until the 29th week. He will have, therefore, 2 aliquots each of 21 specimens to store.

7/16/74	pre-surgery	1-0005-100
7/24/74	week 1	1-0005-101
7/31/74	2	1-0005-102
8/14/74	4	1-0005-103
8/28/74	6	1-0005-104
9/10/74	8	1-0005-105
9/25/74	10	1-0005-106
10/ 9 74	12	1-0005-107
10/30/74	15	1-0005-108
11/22/74	18	1-0005-109
12/ 2/74	20	1-0005-110
12/10/74	21	1-0005-111
12/17/74	22	1-0005-112
12/23/74	23	1-0005-113
12/30/74	24	1-0005-114
1/ 7/75	25	1-0005-115
1/14/75	26	1-0005-116
1/21/75	27	1-0005-117
1/28/75	28	1-0005-118
2/ 4/75	29	1-0005-119
4/22/75	40	1-0005-120

Specimens 1-0002-100 through 1-0002-117 from patient 1-0002 are stored in PATIENT BOX 1-1, compartments 2-A through 2-D. This space is filled on 12/12/74. Specimens 1-0005-100 through 1-0005-117 are stored in PATIENT BOX 1-1, compartments 5-A through 5-D. That space is filled on 1/21/75.

Specimen 1-0002-118, collected on 1/3/75, would be the first filed in SPECIAL BOX 1-1. The two aliquots would be placed in positions 1 and 2 of compartment 1-A. The next specimen would be 1-0005-118, collected on 1/28/75. Those two aliquots would go in positions 3 and 4 of compartment 1-A. The next specimen is 1-0005-119, collected on 2/4/75, and its aliquots would go in positions 5 and 6, compartment 1-A. The next in terms of collection chronology would be 1-0002-119 on 4/15/75, with aliquots into positions 7 and 8 of 1-A. Finally in terms of this example, 1-0005-120, collected on 4/22/75, would have one aliquot in position 9, 1-A, and one aliquot in position 1 of 1-B. This arrangement is illustrated in FIGURE 4.

13. The DONOR BOX separates donor specimens because of the unpredictable variability in their number (from one to 16). The DONOR BOXES should be filled sequentially 1-A, 1-B, 1-C, 1-D, 2-A, 2-B. . . . 6-D. The placing of specimen vials should be left to right, row by row.

    For example, we will assume at institution #1 the first two patients are controls and the third is the recipient of three units of blood. There would be two aliquots each labelled 1-0003-201, 1-0003-202, and 1-0003-203, to be placed as shown in FIGURE 5. The next patient is a control, and the fifth (1-0005) a recipient of five units. The two aliquots of 1-0005-201 and one aliquot of 1-0005-202 go in the third row of compartment 1-A, and the second aliquot of 1-0005-202 as well as the two aliquots of 1-0005-203, 1-0005-204, and 1-0005-205 go into compartment 1-B. If patient 1-0003 now receives another unit, the two aliquots of 1-0003-204 go into the two remaining spaces of 1-B. If patient 1-0001, a control, is then transfused with one unit, becoming thereby a recipient instead of a control, the two aliquots of 1-0001-201 go in the first two positions of compartment 1-C.

14. In packing the specimens, place 2" x 2" gauze pads underneath, on each side, and over the tops of specimen vials in each compartment. The specimens will then be much better protected not only during shipment but also in handling for specimen retrieval at the repository.

Figure 4

Special Box (see item #12, III-10, 13, 14)

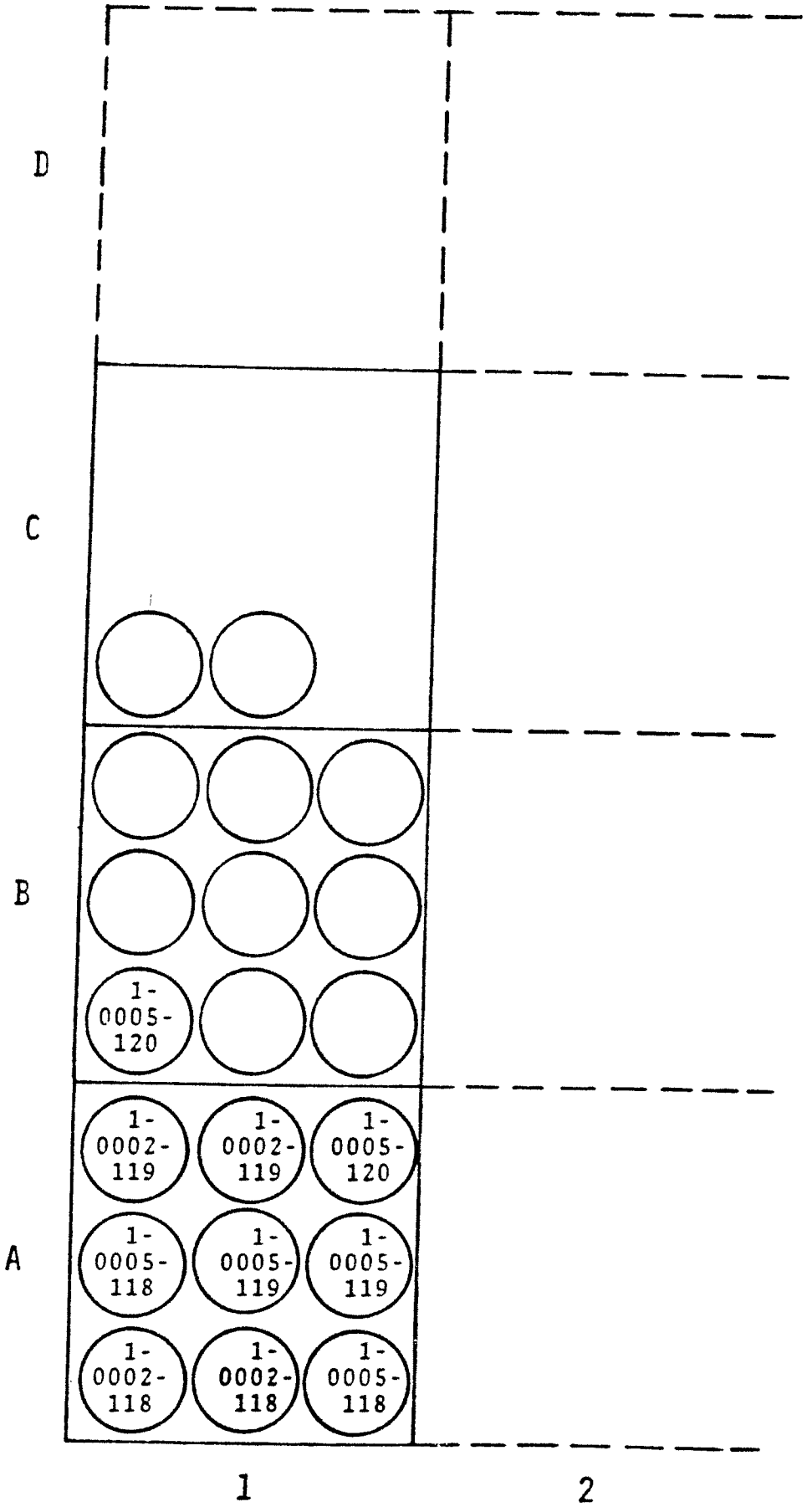
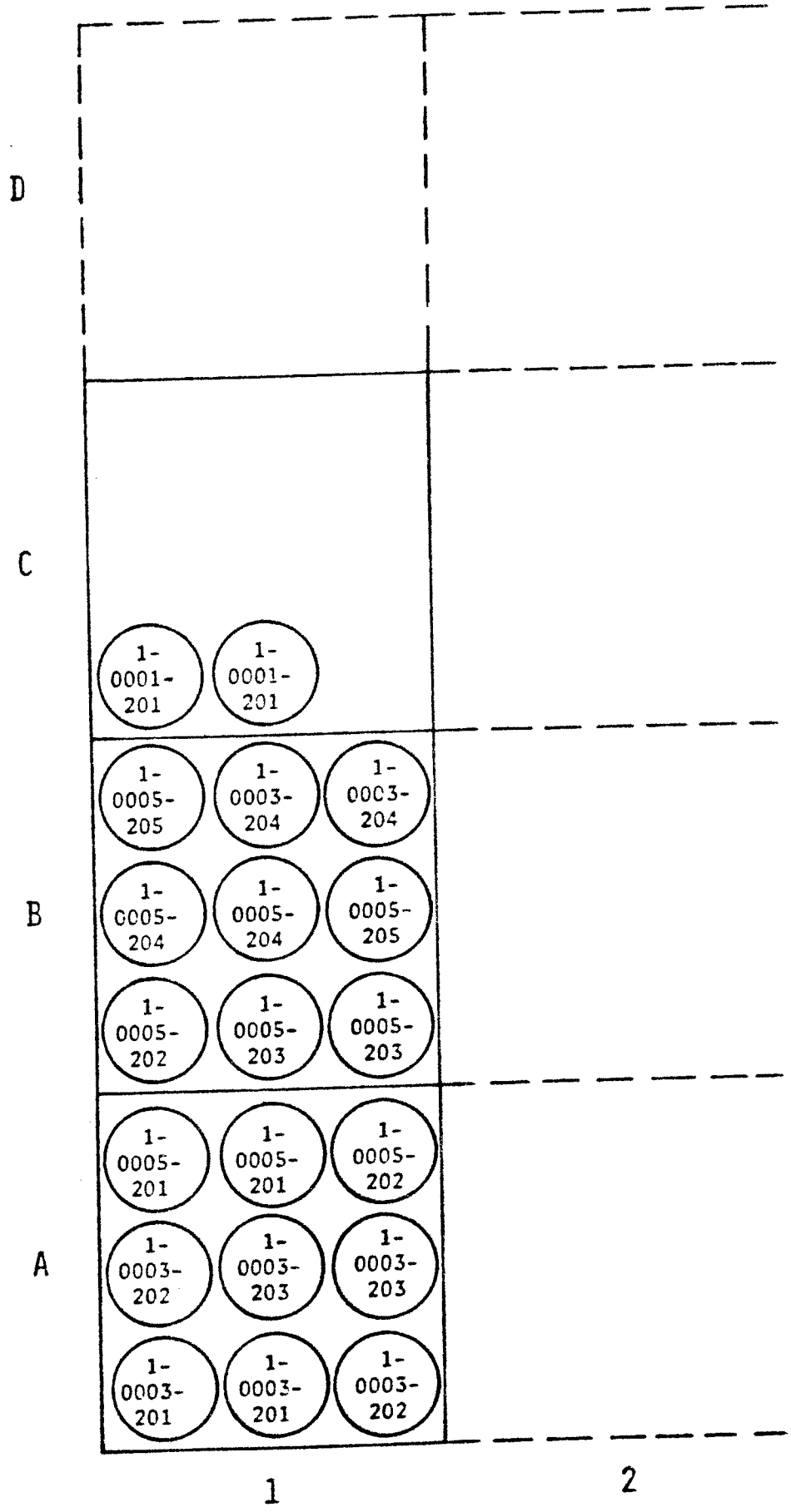


Figure 5
Donor Box (see item #13, III-14)



15. Each patient should bave a PATIENT SPECIMEN LOG. The Front side (FIGURE 6), which is completed by the participating institution represents the record of the location of specimens at the repository. It is retained by the institution until all patient specimens have been completed. The original, with its spaces for recording withdrawals from the repository (FIGURE 7), is then sent to the repository with the patient box containing the patient specimens. The donor specimens, including special follow-up as an implicated or control donor, should already have been collected and shipped to the repository prior to the 40-week (280 days) specimen for the patient.

16. If specimens, either patient or donor, are lost because of spillage or breakage, or there is some other reason for their absence from the set for the repository, note of this should be made under the heading "Comments."

17. Examples of data entries on the PATIENT SPECIMEN LOG are given for fictitious patients 1-0001 through 1-0005 on pages III-27 through III-31. These should be studied periodically to be certain that your data entries conform to the standard. The specimens are likely to be used for many years after the Study is completed, and uniformity is requisite for intelligibility.

18. Whenever a significant event occurs which is related to a patient whose sera has been sent to the repository (an event such as a lab result suggestive of a mislabeled specimen, or an event which has rendered the patient ineligible for inclusion in the study), this should be entered in the comments section of the participating institution's Patient Specimen Log, and a copy of the revised log sent to the repository. The specific nature of the event and the specimen(s) involved must be noted.

Figure 6

Patient Specimen Log
(see item #15, III-17)

TRANSFUSION-TRANSMITTED VIRUSES

PATIENT SPECIMEN LOG

INSTITUTION # ______________ LAST NAME ______________ FIRST ______________

PATIENT STUDY ID ______________ CATEGORY: RECIPIENT [ ] HEPATITIS FOLLOW-UP: YES [ ]

CONTROL [ ] NO [ ]

PATIENT SPECIMENS

____-1__	thru	____-1__	__/__/__	thru	__/__/__	Pt__-__	C__-__-Po__	thru C__-__	Po__
____-1__	thru	____-1__	__/__/__	thru	__/__/__	S__-__	C__-__ Po__	thru C__-__	Po__
____-1__	thru	____-1__	__/__/__	thru	__/__/__	S__-__	C__-__ Po__	thru C__-__	Po__
____-1__	thru	____-1__	__/__/__	thru	__/__/__	S__-__	C__-__ Po__	thru C__-__	Po__
____-1__		____-1__	__/__/__		__/__/__	S__-__	C__-__ Po__	C__-__	Po__
____-1__		____-1__	__/__/__		__/__/__	S__-__	C__-__ Po__	C__-__	Po__

DONOR SPECIMENS

____-2__	thru	____-2__	__/__/__	thru	__/__/__	D__-__	C__-__-Po__	thru C__-__	Po__
____-2__	thru	____-2__	__/__/__	thru	__/__/__	D__-__	C__-__ Po__	thru C__-__	Po__
____-2__	thru	____-2__	__/__/__	thru	__/__/__	D__-__	C__-__ Po__	thru C__-__	Po__
____-2__	thru	____-2__	__/__/__	thru	__/__/__	D__-__	C__-__ Po__	thru C__-__	Po__
____-2__		____-2__	__/__/__		__/__/__	D__-__	C__-__ Po__	C__-__	Po__
____-2__		____-2__	__/__/__		__/__/__	D__-__	C__-__ Po__	C__-__	Po__

COMMENTS ____

PT = PATIENT BOX S = SPECIAL BOX D = DONOR BOX

C = COMPARTMENT Po = POSITION

Figure 7

Record of Reserve Use
(see item #15, III-17)

RECORD OF RESERVE USE

DATE OF WITHDRAWAL	SPECIMEN NUMBER	AMOUNT USED	TO WHOM ALLOCATED	COMMENT

III-C. SHIPMENT OF NIH SPECIMENS TO FLOW LABORATORIES

A. GENERAL CONSIDERATIONS:

1. Ship PATIENT BOXES only when all specimens for the six patients are complete.

2. Patient boxes should be accompanied by the original of the PATIENT SPECIMEN LOG of each patient set in the shipment.

3. Make three copies of each PATIENT SPECIMEN LOG sent. The distribution of the copies is:

   a. One to the Project Officer, TTVS; Division of Blood Diseaseas and Resources, Building 31A, NHLBI, NIH, Bethesda MD

   b. One to the Coordinating Center, USC

   c. One for retention by the institution

4. Ship DONOR BOXES whenever they are full. Thus, donor specimens for any patient will be sent well in advance of shipment of the patient's specimens. Even specimens for implicated and control donor follow-ups will probably be sent in advance because the occurrence of hepatitis will usually be known during the first six months of follow-up, and patient specimens are not complete until the 10-month specimen is obtained. The exception will be HBV infections diagnosed only by late seroconversions.

5. Ship SPECIAL BOXES whenever one is full. These are likely, therefore, to go to the repository many months after the first 18 patient specimens, because they will be used only for patients having 19 or more collections.

6. For each shipment, complete the four-copy (NCR) form RECORD OF SPECIMEN SHIPMENT. The destination of the four sets is marked on the bottom of the form (Figure 1).

Figure 1
(see item #6, III-25)

RECORD OF SHIPMENT TO NIH SPECIMEN REPOSITORY

TRANSFUSION-TRANSMITTED VIRUSES STUDY

Institution_____

Date sent to repository_____

Boxes sent (List individually)

Box designation	Inclusive patient ID's	Comments
P		
P		
P		
P		
P		
P		
P		
P		
P		
P		
D		
D		
D		
D		
D		
S		
S		

a. The original (white) accompanies the shipment to Flow Laboratories.

b. The first copy (canary) is for the institution's records.

c. The second copy (pink) goes to the Project Officer, DBDR, NHLBI.

d. The third copy (goldenrod) goes to the coordinating center (USC).

B. ACTUAL SHIPMENT

Human blood and all derivatives are considered "etiologic agents" of disease under federal regulations covering interstate shipment. It is very necessary, therefore, to conform to these rules in view of the potential jeopardy to our Study if we happened to be responsible for an "incident."

Packing should be carried out in the cold to avoid breakage from rapid temperature changes. If this is not possible, the boxes should be transferred to an ordinary freezer (-10° to -30°C) for several hours prior to handling.

1. Specimen box

a. As indicated on page III-21, 2" x 2" gauze squares should be packed in the boxes as compartments are filled to prevent breakage in handling at the institution. Check to see that this has been done. The material helps in conforming to the requirement that there be enough absorbent in the package to handle the entire fluid volume, as well as preventing breakage in shipment.

b. Wrap the box in Pampers or a material equivalent in absorbency and cushioning effect. Secure the material with rubber bands.

c. Place the wrapped box in a heavy duty plastic bag that can be heat-sealed or made water-tight in some other way.

2. Shipping containers

   a. Place dry ice on the bottom.

   b. Place the wrapped and sealed specimen boxes on the dry ice, and then add dry ice about the sides.

   c. Add enough cushioning to prevent drastic shifting as the ice evaporates.

   d. On the outside securely place the following labels:

      i. Your name, address, and telephone number.

      ii. Address as follows:

          Flow Laboratories, Inc.
          7655 Old Springhouse Road
          McLean, VA 22102

          Attn: Repository
                Mr. Rodney Miller

      iii. Add the following two labels to the outside of the package:

          MEDICAL MATERIALS
          CONFORMS WITH FEDERAL STANDARD
          49 CFR 173, 387; 42 CFR 72.25 (c);
          AND NIH GUIDE, February 10, 1975

e. Final instructions.

i. Ship only on Monday or Tuesday.

ii. Use only Federal Express as the shipping agent.

iii. Obtain the following information:

a. Waybill number

b. The airline to be used

c. The flight number

iv. Call Mr. Rodney Miller or one of his associates in the repository: (703) 893-5925. Provide him with the above information, and also the number of boxes being sent.

C. INVENTORY AT THE REPOSITORY

1. A form (Figure 2) has been designed for each institution's boxes received at the repository.

2. The updated inventory list will be duplicated each month at Flow Laboratories, with one copy being sent to the Project Officer, NHLBI, and a second copy to the coordinating center at USC.

Figure 2
(see item C-1, III-29)

SPECIMEN INVENTORY

TRANSFUSION-TRANSMITTED VIRUSES STUDY

Institution_____

Box Designation	Date Received	Comments

IV. DATA MANAGEMENT SYSTEM

IV. DATA MANAGEMENT SYSTEM

A. COMPUTERIZATION OF STUDY DATA

COMPUTER PROGRAMS

Procedure for the collection of data for this study includes the computerization of patient data directly by the Participating Unit, rather than the mailing of completed forms to the Coordinating Center for accession of data.

The Coordinating Center maintains a set of data files for each Participating Unit. A separate computer program is used in accessing data from each type of form. It is a narrative program which follows the sequence of items on the individual forms but uses abbreviated phrases. There are so-called edit checks at critical stages, designed to assure accuracy of the information accessed.

COMPUTER FILES

FILE DESIGN

The TTV Study utilizes a number of different computer files, that is, mechanisms for storage of the data.

DATA FILES - DATA ACQUISITION

Two principal types of file construction are used:

1. Direct Access Binary Files

   Enables access and use of a data record (space for storage of a defined set of characteristics). All data stored are first translated to patterns of 1's and 0's (the binary representation of each datum). This file type is used when the data record is fixed in size and there is continuing need to use the defined record.

2. Sequential Binary Files

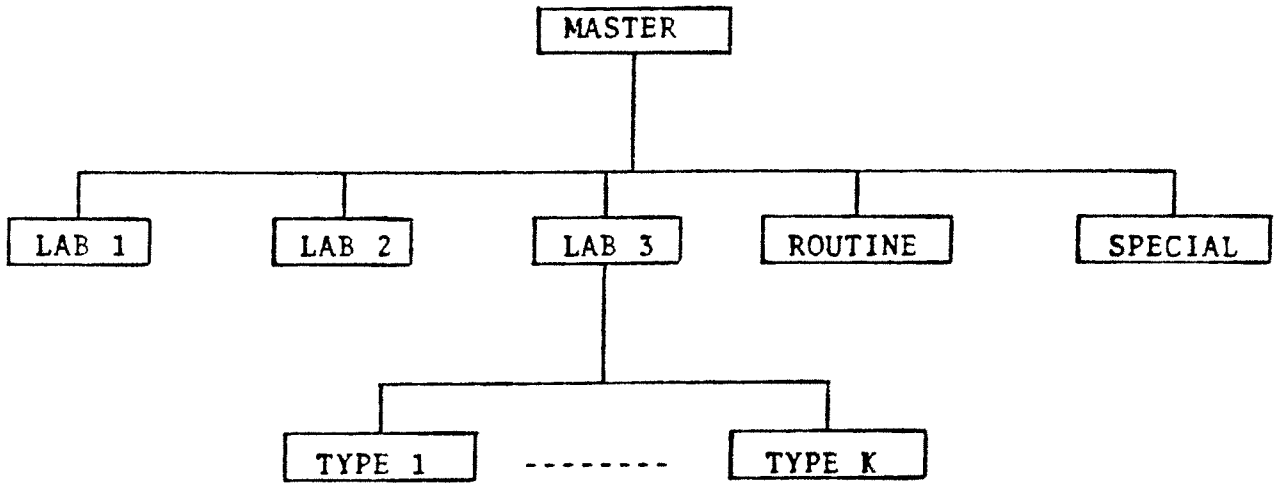
   Provides for the sequence of data records unrestricted in terms of specific identifying characteristics. Thus, a variable number of records for any individual may be stored in any order. This file is used when the data records are being accumulated without regard to individual identifiers. Records are added to the bottom of the file, thus allowing for dynamic growth of the file. When used with the Data Acquisition Program, these data files provide a temporary repository until the data records are transferred to the master files. In keeping with this transitory storage, the sequential binary files are called 'holding' files.

B. THE MASTER FILE STRUCTURE

This set of files and operating programs constitute the heart of the file system. The basic design is user-controlled but assumes a set of data record types reflecting some hierarchy of information associated with studying the subject.

The specific file system is stored in a binary file called the logic file. The data records making up the actual study process are stored in physical files of the sequential binary type. The operating programs maintain the bookkeeping required to locate, store and retrieve specified records. The user is not involved in this process once the file design has been established in the logic file. The structures used in the TTV Study are shown below.

MASTER FILE STRUCTURE FOR PATIENTS



LAB 1: Antigen; ALT

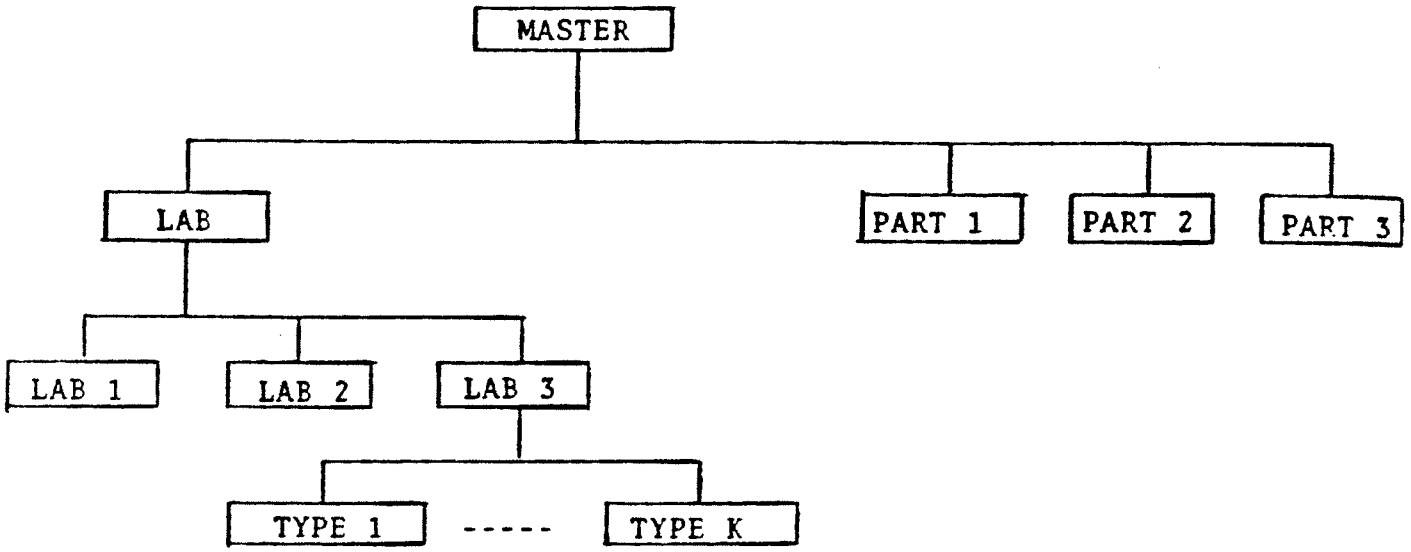
LAB 2: RIA; PHA; Subtype

LAB 3: Special tests
Type 1, ---, Type K; where K=10

ROUTINE: Clinic data collected using protocol schedule

SPECIAL: Clinic data collected at special visits

MASTER FILE STRUCTURE FOR DONORS



LAB: Same as patient

PART 1: Hospital data

PART 2: Collection agency data

PART 3: Special follow-up data

C. DATA ACQUISITION - MASTER FILE SUPPORT FILES

Additional files are used to provide instructions to either the computer programs or to the specific user. These files are prepared in ASCII form (human readable) and are stored as either direct access or sequential binary for long term use.

DIRECT ACCESS INSTRUCTION FILES - TEXTFILES

These are used in conjunction with the data acquisition program. The instructions include messages to the user and criteria for editing and storing responses from the user. A program, THE TEXT GENERATOR, prepares the direct access binary version of the textfile. Once the textfile is prepared, the ASCII version should be stored in archival storage.

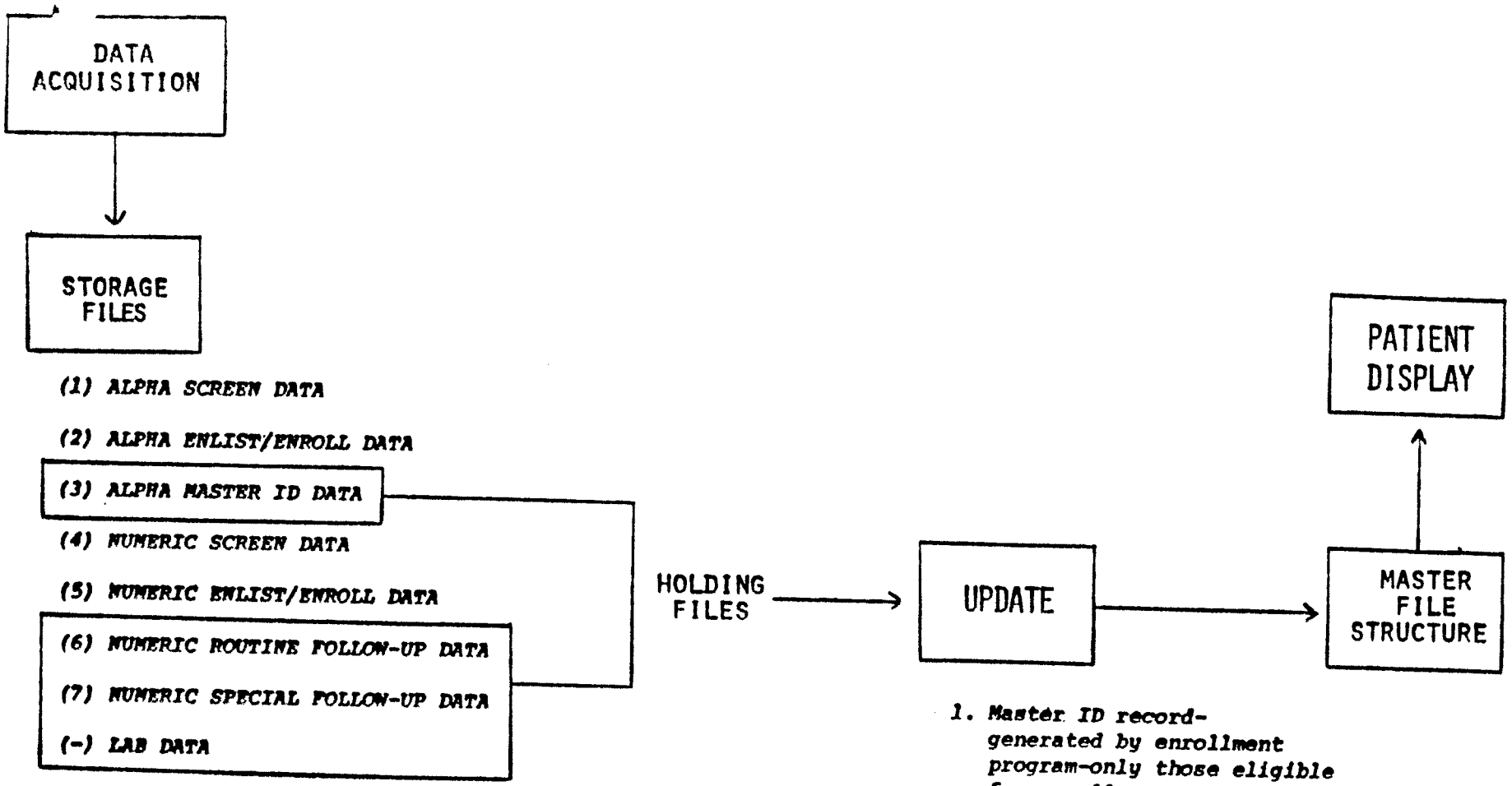
SEQUENTIAL BINARY INSTRUCTION FILES - CONTROL FILES

These are used in conjunction with both the data acquisition and master file programs. The instructions are primarily directed toward provision of user understanding in review of reports prepared by the computer programs. Examples are:

1. Names of characteristics comprising a data record and names of attributes for each characteristic

2. Instructions dealing with selection, edit and display of records from the master file structure

Construction of each file is associated with answering pre-identified questions. These answers constitute the instruction set for a specific task.



1. *Master ID record-generated by enrollment program-only those eligible for enrollment.*
2. *Routine follow-up records-for those patients whose master ID record has been updated.*
3. *Special follow-up records-for those patients whose master ID record has been updated.*
4. *Lab records-for those patients whose master ID record has been updated.*

DESCRIPTION OF CONTROL FILE

POSITION	NAME OF FILE	# CASES	# WORDS/VARIATES	FILE TYPE
(1)	ALPHA DATA SCREEN FILE	—	18	SEQB
(2)	ALPHA DATA ENLIST/ENROLL FILE	--	33	RAND
(3)	ALPHA DATA MASTER ID FILE	—	20	SEQB
(4)	NUMERIC DATA SCREEN FILE	--	23	SEQB
(5)	NUMERIC DATA ENLIST/ENROLL FILE	--	29	RAND
(6)	NUMERIC DATA ROUTINE FOLLOW-UP FILE	--	15	SEQB
(7)	NUMERIC DATA SPECIAL FOLLOW-UP FILE	--	18	SEQB

The control file contains all data files used by the data acquisition programs. Each file is assigned a position in the control file. Whenever a record is generated by use of a data acquisition program, the number of cases for the appropriate file is increased accordingly.

ILLUSTRATION OF RELATIONSHIP BETWEEN A VARIATE, RECORD, AND DIRECT ACCESS BINARY DATA FILE

(1)

DESCRIPTION OF VARIATES IN NUMERIC FILE FOR ENLISTMENT/ENROLLMENT

01,-CATEGORY SURGERY	02	02
02,-MONTH SCREEN	03	04
03,-DAY SCREEN	03	05
04,-SEX	06	06
05,-AGE	06	07
06,-MONTH SURG. SCHED.	15	54
07,-DAY SURG. SCHED.	15	55
08,-SGPT DONE	07	15
09,-UNITAGE-SGPT	07	16
10,-SGPT VALUE	07	17
11,-SGOT DONE	07	20
12,-UNITAGE-SGOT	07	22
13,-SGOT VALUE	07	23
14,-OPERATION	03	04
15,-MONTH OPERATION	03	05
16,-DAY OPERATION	03	06
17,-ANESTHESIA	03	22
18,-TRANSFUSED	04	09
19,-NUMBER UNITS	04	10
20,-REASON TRANSFUSED	04	11
21,-PRIOR TRANSFUSION	07	17
22,-REASON SCREENED	01	46
23,-NUMBER PRIOR UNITS	07	19
24,-AGE PRIOR TRANSF.	07	20
25,-REASON INELIGIBLE	08	27
26,-MONTH REF. DATE	09	33
27,-DAY REF. DATE	09	35
28,-YEAR REF. DATE	09	35
29,-STATUS IN STUDY	09	36

(2)

POSITION OF VARIATE IN DATA RECORD

STUDY ID NO.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	
168.	5.	3.	24.	1.	66.	3.	25.	
1.	1.	0.	0.	0.	0.	1.	3.	(0-15)
25.	2.	1.	0.	0.	3.	1.	0.	(16-23)
0.	0.	3.	25.	75.	2.			(24-29)

(3)

POSITION OF RECORD IN DIRECT ACCESS BINARY DATA FILE

166.00	5.00	3.00	20.00	1.00	69.00	3.00	21.00
1.00	1.00	15.00	0.	0.	0.	1.00	3.00
21.00	2.00	2.00	0.	0.	3.00	1.00	0.
0.	0.	3.00	21.00	75.00	2.00		
167.00	1.00	3.00	19.00	2.00	60.00	3.00	20.00
1.00	1.00	4.00	0.	0.	0.	1.00	3.00
20.00	2.00	1.00	11.00	1.00	3.00	1.00	0.
0.	0.	3.00	20.00	75.00	1.00		
168.00	5.00	3.00	24.00	1.00	66.00	3.00	25.00
1.00	1.00	4.00	0.	0.	0.	1.00	3.00
25.00	2.00	1.00	0.	0.	3.00	1.00	0.
0.	0.	3.00	25.00	75.00	2.00		
169.00	0.00	3.00	24.00	1.00	61.00	0.	0.
1.00	1.00	14.00	0.	0.	0.	1.00	3.00
25.00	2.00	0.	0.	0.	3.00	1.00	0.
0.	1.00	0.	0.	0.	0.		
170.00	1.00	3.00	24.00	1.00	65.00	3.00	25.00
1.00	1.00	5.00	0.	0.	0.	1.00	3.00
25.00	2.00	0.	14.00	1.00	3.00	1.00	0.
0.	0.	3.00	25.00	75.00	1.00		

ILLUSTRATION OF RELATIONSHIP BETWEEN A VARIATE, RECORD, AND SEQUENTIAL BINARY DATA FILE

(1)

DESCRIPTION OF VARIATES IN NUMERIC FILE FOR ROUTINE FOLLOW-UP

01,-DATE (YYMMDD)	04	04
02,-PLACE	06	06
03,-OPERATION	07	08
04,-TRANSFUSED	08	09
05,-EXPOSED-HEPATITIS	09	12
06,-GLOBULIN	09	13
07,-INH	10	14
08,-ALDOMET	10	05
09,-HEALTH	11	15
10,-SYMPTOMS	11	16
11,-TIRING	12	17
12,-APPETITE	12	18
13,-JAUNDICE	13	19
14,-HEPATITIS SUSPECTED	13	20
15,-ELIGIBILITY	14	22

(2)

POSITION OF VARIATE IN DATA RECORD

STUDY ID NO. (1)	(2)	(3)	(4)	(5)	(6)	(7)	
246. 751125.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	(8-15)

(3)

POSITION OF RECORD IN SEQUENTIAL BINARY DATA FILE

243. 751203.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	
253. 751203.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	
238. 751203.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	
246. 751125.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	
244. 751125.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	
311. 751203.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	
245. 751203.	4.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	
241. 751203.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	
242. 751202.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	
237. 751202.	2.	2.	2.	2.	0.	2.	
2. 2.	0.	0.	0.	0.	0.	1.	

NAME FILE FOR DATA ACQUISITION PROGRAMS

The "name" file describes all variates or words stored in each data file that appears in the control file. Each data file is identified in the "name" file by its position in the control file and the number of items it will contain. (In the case of a numeric file, each variate is considered as an item. For an alphameric file, one word or several words can be used as an item.) For each item, there follows a brief description, an item number, and a program number. The "name" file lists all files in the same order as they appear in the control file.

An example of a numeric file :

6 15 *(6th position-ROUTINE FOLLOW-UP FILE, 15 items in file.)*

01,-DATE (YYMMDD)	04	04
02,-PLACE	06	06
03,-OPERATION	07	08
04,-TRANSFUSED	08	09
05,-EXPOSED-HEPATITIS	09	12
06,-GLOBULIN	09	13
07,-INH	10	14
08,-ALDOMET	10	05
09,-HEALTH	11	15
10,-SYMPTOMS	11	16
11,-TIRING	12	17
12,-APPETITE	12	18
13,-JAUNDICE	13	19
14,-HEPATITIS SUSPECTED	13	20
15,-ELIGIBILITY	14	22

(in this file the items correspond to the variate numbers)

An example of an alphameric file:

```
3 8        (3rd position-MASTER ID FILE, 8 items in file)
01,06,-NAME                    02  45   (item 1 will be words 1-6)
07,12,-ADDRESS                 10  46   (item 2 will be words 7-12)
13,14,-PHONE                   10  47   (item 3 will be words 13-14)
15,15,-DAY-VISIT               10  48   (item 4 will be word 15)
16,16,-TIME-VISIT              10  49   (item 5 will be word 16)
17,17,-LOCATION-HORIZONTAL     10  50   (item 6 will be word 17)
18,18,-LOCATION-VERTICAL       10  51   (item 7 will be word 18)
19,20,-ENROLL (YYMMDD)         09  33   (item 8 will be words 19-20)
```

A complete listing of the "name" file follows.

```
1 1
13,18,-CATEGORY SURGERY    02  03
2 10
01,05,-LAST NAME           15  49
 6,07,-FIRST NAME          15  50
08,10,-HOSPITAL CHART NO.  15  51
11,12,-ZIPCODE             15  52
13,18,-CATEGORY SURGERY    02  03
19,21,-OPERATION*          03  08
22,24,-REASON TRANSFUSED*  04  12
25,27,-MAJOR DIAGNOSIS*    05  14
28,30,-PERTINENT DIAG.*    06  16
31,33,-REASON INELIG.*     08  28
3 8
01,06,-NAME                02  45
07,12,-ADDRESS             10  46
13,14,-PHONE               10  47
15,15,-DAY-VISIT           10  48
16,16,-TIME-VISIT          10  49
17,17,-LOCATION-HORIZONTAL 10  50
18,18,-LOCATION-VERTICAL   10  51
19,20,-ENROLL (YYMMDD)     09  33
4 23
01,-CATEGORY SURGERY     02  02
02,-MONTH SCREEN         03  04
03,-DAY   SCREEN         03  05
04,-SEX                  06  06
05,-AGE                  06  07
06,-NORMAL SGPT          07  18
07,-NORMAL SGOT          07  24
08,-SGPT DONE            07  15
09,-UNITAGE-SGPT         07  16
10,-SGPT VALUE           07  17
11,-SGOT DONE            07  20
12,-UNITAGE-SGOT         07  22
13,-SGOT VALUE           07  23
14,-HISTORY-HEPATITIS    08  26
15,-TRANSFUSED           10  27
16,-EXPOSED-HEPATITIS    11  28
17,-WORK EXPOSURE        12  29
18,-AGE-UNDIAG. JAUND.   09  48
19,-UNDIAG. JAUNDICE     09  32
20,-CONSENT REQUESTED    13  33
21,-CONSENT SIGNED       14  34
22,-REASON SCREENED      01  46
23,-AGE-HEPATITIS        08  47
5 29
01,-CATEGORY SURGERY     02  02
02,-MONTH SCREEN         03  04
03,-DAY   SCREEN         03  05
04,-SEX                  06  06
05,-AGE                  06  07
06,-MONTH SURG. SCHED.   15  54
07,-DAY   SURG. SCHED.   15  55
08,-SGPT DONE            07  15
09,-UNITAGE-SGPT         07  16
10,-SGPT VALUE           07  17
```

11,-SGOT DONE	07	20
12,-UNITAGE-SGOT	07	22
13,-SGOT VALUE	07	23
14,-OPERATION	03	04
15,-MONTH OPERATION	03	05
16,-DAY OPERATION	03	06
17,-ANESTHESIA	03	22
18,-SUBTYPE TO PHA	04	09
19,-NUMBER UNITS	04	10
20,-REASON TRANSFUSED	04	11
21,-PRIOR TRANSFUSION	07	17
22,-REASON SCREENED	01	46
23,-NUMBER PRIOR UNITS	07	19
24,-AGE PRIOR TRANSF.	07	20
25,-REASON INELIGIBLE	08	27
26,-MONTH REF. DATE	09	33
27,-DAY REF. DATE	09	35
28,-YEAR REF. DATE	09	35
29,-STATUS IN STUDY	09	36
6 15		
01,-DATE (YYMMDD)	04	04
02,-PLACE	06	06
03,-OPERATION	07	08
04,-TRANSFUSED	08	09
05,-EXPOSED-HEPATITIS	09	12
06,-GLOBULIN	09	13
07,-INH	10	14
08,-ALDOMET	10	05
09,-HEALTH	11	15
10,-SYMPTOMS	11	16
11,-TIRING	12	17
12,-APPETITE	12	18
13,-JAUNDICE	13	19
14,-HEPATITIS SUSPECTED	13	20
15,-ELIGIBILITY	14	22
7 18		
01,-DATE (YYMMDD)	04	04
02,-REASON	05	06
03,-MALAISE	06	07
04,-NAUSEA	06	08
05,-VOMITING	06	09
06,-BROWN (DARK) URINE	06	11
07,-JOINT PAIN	06	12
08,-RASH	06	13
09,-BILIRUBIN	13	21
10,-ITCHING	06	14
11,-ABDOMINAL PAIN	06	10
12,-1ST SX (YYMMDD)	06	22
13,-JAUNDICE	07	15
14,-ACTIVITY	08	16
15,-HOSPITALIZATION	10	18
16,-PHYSICIAN	09	17
17,-BIOPSY DONE	11	19
18,-BIOPSY RESULT	11	20

The following is an actual copy of the "Menu" of Programs. This is the first entry to appear when you have entered your codes and are ready to begin accessing data.

```
*****************************************
*****************************************
  DATA ACQUISITION SYSTEM
        TTV     STUDY


YOUR LAST NAME ----?

UPDATE --- TOMORROW
PROGRAM (? FOR LIST)----??

PROGRAM TO BE USED:

1 = SCREEN
2 = ENLIST
3 = ENROLL
4 = ROUTINE FOLLOW-UP
5 = SPECIAL FOLLOW-UP
6 = LAB (ANTIGEN & SGPT)
7 = DONOR DATA
8 = MESSAGE
9 = STOP SYSTEM
ADDITIONAL PROGRAMS:
10= UPDATE REQUEST
11= LIST UPDATE DISPLAY
12= ENTER QUALITY CONTROL DATA
13= ENTER MESSAGE TO COORDINATING CENTER

PROGRAM (0 FOR LIST)----?1
```

Messages from the Coordinating Center appear here.

Authorized persons are allowed to continue.

Programs available to the institutions.

Upon entry, the computer operation is controlled by the Monitor. At completion of each available program, control is returned to the Monitor. The process continues until selection of Program 9. This institutes updating of computer use charges and termination of the telephone connection. This control function is designed to assist novice users in easily accomplishing required tasks. As such, the process is both efficient and economical.

DESCRIPTION OF UPDATE PRINTOUT

(1)
```
 12/05/75
UPDATE ENROLLMENT
GOLUB                          ASSIGNED ID #, 363

PROGRAM STOP AT 2000
```

(all patients enrolled since last update will be reported here)

(2)
```
SORT ROUTINE VISIT RECORDS
TOTAL USED     4.52 UNITS
          51 RECORDS READ
          51 RECORDS WRITTEN
           0 RECORDS DELETED
SORT SPECIAL VISIT RECORDS
TOTAL USED     6.37 UNITS
           1 RECORDS READ
           1 RECORDS WRITTEN
           0 RECORDS DELETED
SORT LAB REPORTS
TOTAL USED     8.73 UNITS
         150 RECORDS READ
         150 RECORDS WRITTEN
           0 RECORDS DELETED
```

All records in the sequential binary data files must be in ID order. If there is more than one record for an ID, they must be in chronological order.

(3)
```
UPDATE ROUTINE VISIT RECORDS
GET ERROR 1          362
NOTIFY COORDINATING CENTER

PROGRAM STOP AT 1030
UPDATE SPECIAL VISIT RECORDS

PROGRAM STOP AT 1010
UPDATE LAB REPORTS
GET ERROR 1          271
GET ERROR 1          362
GET ERROR 1          362
GET ERROR 2          271
GET ERROR 2          362
GET ERROR 2          362
GET ERROR 3          271
GET ERROR 3          362
GET ERROR 3          362
PLEASE NOTIFY COORDINATING CENTER

PROGRAM STOP AT 2120
```

Records are reported here if ID is not in master file structure, or if there is a duplicate record (lab update will accept duplicate records, routine and special update won't).

(4) RECORDS UNACCEPTABLE IN UPDATE
ROUTINE VISITS:
END OF ROUTINE VISITS

SPECIAL VISITS:
END OF SPECIAL VISITS

Records that were not updated into master file structure will appear here. These records are "lost" and must be recovered by re-entry through the appropriate data acquisition program.

LAB RECORDS.

271.	2	751113	241
336.	2	751112	25
358.	1	751127	10
358.	2	751127	19
362.	1	751129	10
362.	2	751129	12

END OF LAB RECORDS

IF NECESSARY, RE-ENTER DATA THROUGH
DATA ACQUISITION PROGRAMS

DATA ACQUISITION FILES REFRESHED

(5) MASTER SCHEDULES FOR ENROLLED SUBJECTS

For each enrolled ID taken through update, a display is printed to aid in planning follow-up visits.

NAME GOLUB
ADDRESS 382-0 AVENIDA CASTELLA

TELEPHONE 5866495
OPTIMUM DAY 0 0
LOCATION 0 0

WEEK YYWW		START DAY YYMMDD	END DAY YYMMDD
7545	*ref date*	751102	751108
7546	*1 week*	751109	751115
7547	*2 weeks*	751116	751122
7549	*4 weeks*	751130	751206
7551	*etc.*	751214	751220
7601		751228	760103
7603		760111	760117
7605		760125	760131
7608		760215	760221
7611		760307	760313
7614		760328	760403
7617		760418	760424
7633	*40 weeks*	760808	760814

FLOW CHART DATA

PATIENT ID-- STLO 10

(1=YES 2=NO 0=UNK/NA

DAYS FROM ENTRY	-1	7	15	29	43	57	71	84	106	126	149	166	200	292
ANTIGEN	9	11	10	12	11	10	8	8	5	9	6	8	8	7
SGPT	83	12	28	22	9	6	25	24	27	21	28	19	65	57
ANTI-HBS RIA	1	0	0	0	0	0	0	0	0	0	0	0	0	1
ANTI-HBS PHA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PLACE	0	0	0	4	4	4	4	1	4	2	2	4	4	0
HOSPITAL-NOT HEP.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPERATION	0	0	0	2	2	2	2	2	2	2	2	2	2	0
TRANSFUSION	0	0	0	2	2	2	2	2	2	2	2	2	2	0
HEPATITIS EXPOSURE	0	0	0	2	2	2	2	2	2	2	2	2	2	0
ISONIAZID	0	0	0	2	2	2	2	2	2	2	2	2	2	0
METHYLDOPA	0	0	0	2	2	2	2	2	2	2	2	2	2	0
HEALTH STATUS	0	0	0	1	1	1	1	1	1	2	2	1	1	0
SX-NOT HEPATITIS	0	0	0	0	0	0	0	0	0	1	1	0	0	0
TIRES EASILY	0	0	0	0	0	0	0	0	0	0	0	0	0	0
APPETITE	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ELIGIBILITY	0	0	0	1	1	1	1	1	1	1	1	1	5	0
REASON	0	0	0	0	0	0	0	0	0	0	0	0	0	2
MALAISE	0	0	0	0	0	0	0	0	0	0	0	0	0	1
NAUSEA	0	0	0	0	0	0	0	0	0	0	0	0	0	2
VOMITING	0	0	0	0	0	0	0	0	0	0	0	0	0	2
BROWN URINE	0	0	0	0	0	0	0	0	0	0	0	0	0	2
[illegible] PAIN	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	0	0	0	0	0	0	0	0	0	0	0	0	0	2
MAX BILIRUBIN	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ITCHING	0	0	0	0	0	0	0	0	0	0	0	0	0	2
ABDOMINAL PAIN	0	0	0	0	0	0	0	0	0	0	0	0	0	2
LENGTH-EPISODE HEP.	0	0	0	0	0	0	0	0	0	0	0	0	1	13
[illegible]	0	0	0	0	0	0	0	0	0	0	0	0	0	2
ACTIVITY	0	0	0	0	0	0	0	0	0	0	0	0	0	1
HOSPITAL-HEPATITIS	0	0	0	0	0	0	0	0	0	0	0	0	0	2
M.D. CONTACTED	0	0	0	0	0	0	0	0	0	0	0	0	0	2

D. TEXTFILES

The textfile is the fundamental mechanism by which data are entered, edited, and stored for this study. A textfile contains text for all questions that appear, the edit instructions for anticipated responses, and the storage instructions for building data records.

A different textfile is used for each segment or phase of the study.

Textfiles are prepared by the textfile generator program, TEXGEN1.

INSTRUCTIONS FOR USING THE TEXT GENERATOR PROGRAM:

STUDY SEGMENT ? *A four character word to designate the segment of the study.*

For each specific question:

QUESTION NUMBER ? *Each set of instructions associated with an item of information is identified by 'Question number?' Use 0 to stop the text generator program.*

NUMBER OF LINES OF TEXT ? *(Maximum = 20; No text = 0)*

For text:

For each line of text:

LINE ... ? *Maximum of 40 characters each line. Text must start within 4 spaces of the line number.*

NUMBER OF SKIPS ? *Controls line feeds before text lines. 0 = line feed before line 1 only.*

For each skip:

LINE FEED BEFORE LINE ?

RESPONSE TYPE ? *Options: 1 = No response*
2 = Alpha response
3 = Numeric response

For alpha response:

TEST TYPE ? *Options: 0 = Write record from core to file*
1 = Load file records into core
2 = Test alpha constant, with or without store
3 = Store alpha data, no test
4 = Chain new program
5 = Describe characteristics unspecified in advance
6 = Transfer alpha words from one file to a second file

For Option 1:

NUMBER OF ALPHA FILES LOADED ?

For each file:

POSITION IN CONTROL FILE ? *Indicate location in the control file list.*

NUMBER OF FILE USED TO ASSIGN ID ? *Location in load list*

For Option 2:

NUMBER OF CONSTANTS ?

POSITION OF FIRST WORD IN THE RECORD ? *0 = No store*

If data to be stored:

POSITION OF LAST WORD ?

NUMBER OF ALPHA FILE ? *Location in control file list*

For each constant:

CONSTANT ... ? *Maximum: 40 characters*

For Option 3:

NUMBER OF FIRST FILE ?

NUMBER OF SECOND FILE ? *0 if not used; if 2 files are used, the data will be stored in the same alpha word locations in both files.*

POSITION OF FIRST WORD IN RECORD ?

POSITION OF LAST WORD IN RECORD ?

For Option 4:

NAME OF NEW PROGRAM ?

STUDY LABEL ? *Four character word to designate the study*

STUDY SEGMENT ? *Four character word to designate the segment of the study*

QUESTION NUMBER TO START ?

For Option 5:

NUMBER OF DATA FILE ? *Indicate location in control file list; stores 1 or 0 for each characteristic*

MAXIMUM NUMBER OF CHARACTERS DESCRIBED ? *Allocates locations in the text file to store descriptions of characteristics to be specified; Max = 100*

NUMBER OF VARIATE IN DATA FILE FOR FIRST CHARACTERISTIC ?

For Option 6:

NUMBER OF ORIGINAL FILE ? *Location in control file list*

POSITION OF FIRST WORD IN ORIGINAL RECORD ?

POSITION OF LAST WORD IN ORIGINAL RECORD ?

NUMBER OF NEW FILE ? *Location in control file list*

POSITION OF FIRST WORD IN NEW RECORD ?

POSITION OF LAST WORD IN NEW RECORD ?

For numeric response:

TEST TYPE ? *Options:* *0 = Write record from core to file*
1 = Load file records into core
2 = Test numeric constant, with or without store
3 = Test values from files, with or without store
4 = Computations
5 = ID number requested (program will automatically ask the question: 'ID number ?')
6 = Define data sets associated with characteristics unspecified in advance

For Option 1:

NUMBER OF NUMERIC FILES LOADED ?

For each file:

POSITION IN CONTROL FILE ? *Indicate location in the control file list*

NUMBER OF FILE USED TO ASSIGN ID ? *Location in load list*

For Option 2:

NUMBER OF TEST OPTIONS ? *See list below*

USE RESPONSE LAST ENTERED ? *(Y = 1; N = 0)*

STORE DATA ? *Options: 0 = No store*
1 = Store

If data to be stored:

NUMBER OF VARIATE ?

NUMBER OF FIRST FILE ? *Location in control file list*

NUMBER OF SECOND FILE ? *0 if not used; if 2 files are used, the data will be stored in the same location in both files*

For each option:

TEST OPTION ? *Options: 1 = Value less than a constant (X < L)*
2 = Value equals a constant (X = L)
3 = Value greater than a constant (X > L)
4 = Value between two constants (L < X < U)
5 = Value outside interval defined by two constants (X < L or X > U)

CONSTANT ? *Define constant specified as 'L' in the list of options. For date testing, in form YYMMDD, use -999999 for today's date*

For test options 4 or 5:

SECOND CONSTANT ? *Define constant specified as 'U' in the list of options. For date testing, in form YYMMDD, use -999999 for today's date*

For Option 3:

USE RESPONSE LAST ENTERED ? *(Y = 1; N = 0)*

STORE DATA ? *Options: 0 = No store*
1 = Store

If data to be stored:

NUMBER OF VARIATE ?

NUMBER OF FILE ? *Location in the control file list*

TEST OPTION ? *Options: 1 = Value equals variate in 'File 1' (see below)*
2 = Value less than or equal to variate in 'File 1'
3 = Value greater than variate in 'File 1'
4 = Value inside interval defined by variates in 'File 1' and 'File 2'
5 = Value outside interval defined by variates in 'File 1' and 'File 2'

NUMBER OF 'FILE 1' ? *Location in the control file list*

NUMBER OF VARIATE IN 'FILE 1' ?

For test options 4 or 5:

NUMBER OF 'FILE 2' ? *May be the same as 'File 1'*

NUMBER OF VARIATE IN 'FILE 2' ?

For Option 4:

USE RESPONSE LAST ENTERED ? *(Y = 1; N = 0)*

STORE DATA ? *Options: 0 = No store*
1 = Store File 1 only
2 = Store File 1 and File 2

If data to be stored:

NUMBER OF FIRST FILE ? *Location in control file list*

NUMBER OF VARIATE IN FIRST FILE ?

If stored in second file:

NUMBER OF SECOND FILE ? *Location in the control file list*

NUMBER OF VARIATE IN SECOND FILE ?

COMPUTATION OPTION ? *Options: 1 = Value plus variate in 'File 1' (see below)*
2 = Value minus variate in 'File 1'
3 = Value times variate in 'File 1' (value must be greater than 0)
4 = Value divided by variate in 'File 1' (variate in 'File 1' must be greater than 0)
5 = Value raised to a power (Value must be greater than 0)
6 = Log of the value (Value must be greater than 0)
7 = Value plus constant
8 = Value times constant (Value must be greater than 0)

For option 1, 2, 3, or 4:

NUMBER OF 'FILE 1' ?

NUMBER OF VARIATE IN 'FILE 1' ?

For option 5:

POWER ?

For options 7 or 8:

CONSTANT ?

For Option 6:

NUMBER OF DATA FILE ?	*Indicate location in control file list; stores the common data set for each characteristic*
NUMBER OF ITEMS IN THE COMMON DATA SET ?	*Include in the count the item describing the characteristic; Max = 25*
MAXIMUM NUMBER OF CHARACTERISTICS DESCRIBED ?	*Allocates locations in the text file to store descriptions of characteristics to be specified; Max = 200*

For each item in the data set:

QUESTION TO BE ASKED ?	*One line of text for each item; maximum 40 characters. Exclude the first item, describing the characteristic; this question will be asked by the program automatically.*

NEXT QUESTION IF SUCCESS ?

This is the number of the next question if the response test has been satisfied

NEXT QUESTION IF FAILURE ?

This is the number of the next question if the response test has not been satisfied

The two question numbers should be the same if no test is performed; the question numbers must be greater than 0 to control the sequence of questions that appear. The following codes may be used instead of question numbers:

-1 = Next question number will be specified by the user
-2 = Chain to next program; data loaded (use with alpha test type = 4)
0 = Chain to next program; no data loaded (use with alpha test type = 4)

```
CATEGORY OF SURGERY:
GIVE CODE (1 - 9) ----  ?3
```

Question 2 in Screen Program.

```
QUESTION NUMBER (0 TO STOP) ----?2

NUMBER OF LINES OF TEXT ----?2

GIVE TEXT:
LINE --              1
 ?CATEGORY OF SURGERY:


LINE --              2
 ?GIVE CODE (1 - 9) ----


NUMBER OF SKIPS ----?1

SKIP BEFORE LINE ----?1

RESPONSE TYPE ----?3

TEST TYPE ----?2

TEST NUMERIC CONSTANTS:
NUMBER OF TEST OPTIONS USED ----?1

STORE INSTRUCTION ----?1

NUMBER OF VARIATE IN RECORD ----?1

NUMBER OF FILE ----?4

NUMBER OF 2ND FILE (0 IF NOT USED) ----?0

OPTION ----?1

CONSTANT ----?8.5

NEXT QUESTION - SUCCESS ----?4

NEXT QUESTION - FAILURE ----?3
```

Instructions to develop Question 2:

1) Ask 'Category of surgery?'
2) Edit response for codes 1-8.
3) Store code in variate 1, file 4.
4) For codes 1-8, go to Question 4; for code 9, go to Question 3.

```
QUESTION NUMBER (0 TO STOP) ----?3

NUMBER OF LINES OF TEXT ----?1

GIVE TEXT:
LINE --              1
 ?TYPE THE CATEGORY ----


NUMBER OF SKIPS ----?0

RESPONSE TYPE ----?2

TEST TYPE ----?3

TEST ALPHA DATA FROM FILE:
NUMBER OF FILE USED ----?1

NUMBER OF 2ND FILE (0 IF NOT USED) ----?

STORE INSTRUCTION ----?2

FIRST WORD -TEST OR STORE ----?13

LAST WORD ----?18

NEXT QUESTION - SUCCESS ----?4

NEXT QUESTION - FAILURE ----?4
```

Instructions to develop Question 3

1) Ask 'Type the category?'
2) Store response in words 13-18, file 1.
3) Go to Question 4.

Question 7 in Screen Program.

```
QUESTION NUMBER (0 TO STOP) ----?7

NUMBER OF LINES OF TEXT ----?1

GIVE TEXT:
LINE --
 ?AGE OF PATIENT ----

NUMBER OF SKIPS ----?

RESPONSE TYPE ----?3

TEST TYPE ----?2

TEST NUMERIC CONSTANTS:
NUMBER OF TEST OPTIONS USED ----?1

STORE INSTRUCTION ----?1

NUMBER OF VARIATE IN RECORD ----?5

NUMBER OF FILE ----?4

NUMBER OF 2ND FILE (0 IF NOT USED) ----?

OPTION ----?3

CONSTANT ----?15.99

NEXT QUESTION - SUCCESS ----?15

NEXT QUESTION - FAILURE ----?8
```

Instructions to develop Question 7:

1) *Ask 'Age of patient?'*
2) *Edit response for age greater than 16.*
3) *Store age in variate 5, file 4.*
4) *For age greater than 16, go to Question 15; for age less than 16, go to Question 8.*

```
QUESTION NUMBER (0 TO STOP) ----?8

NUMBER OF LINES OF TEXT ----?2

GIVE TEXT:
LINE --              1
 ?YOU HAVE SAID PATIENT IS LESS


LINE --              2
 ?THAN 16 YEARS:  Y OR N ----

NUMBER OF SKIPS ----?0

RESPONSE TYPE ----?2

TEST TYPE ----?2

TEST ALPHA CONSTANTS:
NUMBER OF CONSTANTS ----?1

POSITION OF FIRST WORD IN ALPHA RECORD ----?0

CONSTANT --              1
 ?N

NEXT QUESTION - SUCCESS ----?7

NEXT QUESTION - FAILURE ----?9
```

Instructions to develop Question 8:

1) *Ask 'Patient less than 16?'*
2) *Edit response for NO (N).*
3) *For NO (N), go to Question 7; for YES (Y), go to Question 9.*

MASTER FILE STRUCTURE SUPPORT PROGRAMS

NAME	FUNCTION
STATUS	DETERMINES NEED FOR REORGANIZATION OF FILE
REFRESH	REORGANIZES FILE
UPDATE	STORES NEW DATA
DISPLAY	PROVIDES UPDATED PATIENT SUMMARIES
CORRECT	PROVIDES MECHANISMS TO CORRECT DATA ITEMS OR ENTIRE RECORDS

SELECT ALL RECORDS FROM MASTER FILE STRUCTURE
ENLIST & ENROLLMENT CHARACTERISTICS
SELECT VARIATES:

VAR —	1 -CATEGORY SURGERY	Y;N ——?Y
VAR —	2 -MONTH SCREEN	Y;N ——?Y
VAR —	3 -DAY SCREEN	Y;N ——?Y
VAR —	4 -SEX	Y;N ——?N
VAR —	5 -AGE	Y;N ——?Y
VAR —	6 -MONTH SURG. SCHED.	Y;N ——?N
VAR —	7 -DAY SURG. SCHED.	Y;N ——?N
VAR —	8 -SGPT DONE	Y;N ——?N
VAR —	9 -UNITAGE-SGPT	Y;N ——?N
VAR —	10 -SGPT VALUE	Y;N ——?Y
VAR —	11 -SGOT DONE	Y;N ——?N
VAR —	12 -UNITAGE-SGOT	Y;N ——?N
VAR —	13 -SGOT VALUE	Y;N ——?Y
VAR —	14 -OPERATION	Y;N ——?N
VAR —	15 -MONTH OPERATION	Y;N ——?N
VAR —	16 -DAY OPERATION	Y;N ——?N
VAR —	17 -ANESTHESIA	Y;N ——?Y
VAR —	18 -SUBTYPE TO PHA	Y;N ——?Y
VAR —	19 -NUMBER UNITS	Y;N ——?Y
VAR —	20 -REASON TRANSFUSED	Y;N ——?N
VAR —	21 -PRIOR TRANSFUSION	Y;N ——?N
VAR —	22 -REASON SCREENED	Y;N ——?N
VAR —	23 -NUMBER PRIOR UNITS	Y;N ——?N
VAR —	24 -AGE PRIOR TRANSF.	Y;N ——?N
VAR —	25 -REASON INELIGIBLE	Y;N ——?N
VAR —	26 -MONTH REF. DATE	Y;N ——?Y
VAR —	27 -DAY REF. DATE	Y;N ——?Y
VAR —	28 -YEAR REF. DATE	Y;N ——?Y
VAR —	29 -STATUS IN STUDY	Y;N ——?Y

FLOW CHART CHARACTERISTICS

SELECT VARIATES:

VAR --	1	DAYS FROM ENTRY	Y;N ----?Y
VAR —	2	ANTIGEN	Y;N ----?Y
VAR —	3	SGPT	Y;N ----?Y
VAR —	4	ANTI-HBS RIA	Y;N ----?Y
VAR —	5	ANTI-HBS PHA	Y;N ----?Y
VAR —	o	PLACE	Y;N ----?N
VAR —	7	HOSPITAL-NOT HEP.	Y;N ----?Y
VAR —	8	OPERATION	Y;N ----?N
VAR —	9	TRANSFUSION	Y;N ----?N
VAR —	10	HEPATITIS EXPOSURE	Y;N ----?Y
VAR —	11	ISONIAZID	Y;N ----?N
VAR —	12	METHYLDOPA	Y;N ----?N
VAR —	13	HEALTH STATUS	Y;N ----?Y
VAR —	14	SX-NOT HEPATITIS	Y;N ----?Y
VAR --	15	TIRES EASILY	Y;N ----?N
VAR —	1o	APPETITE	Y;N ----?N
VAR —	17	ELIGIBILITY	Y;N ----?Y

VAR —	18 REASON	Y;N ——?N
VAR —	19 MALAISE	Y;N ——?N
VAR —	20 NAUSEA	Y;N ——?N
VAR —	21 VOMITING	Y;N ——?N
VAR --	22 BROWN URINE	Y;N ——?N
VAR —	23 JOINT PAIN	Y;N ——?N
VAR —	24 RASH	Y;N ——?N
VAR —	25 MAXIMUM BILIRUBIN	Y;N ——?N
VAR --	26 ITCHING	Y;N ——?N
VAR —	27 ABDOMINAL PAIN	Y;N ——?N
VAR —	28 LENGTH-EPISODE HEP.	Y;N ——?Y
VAR —	29 JAUNDICE	Y;N ——?N
VAR --	30 ACTIVITY	Y;N ——?N
VAR —	31 HOSPITAL-HEPATITIS	Y;N ——?Y
VAR —	32 M.D. CONTACTED	Y;N ——?N

VARIATES STORED IN FILE — RETR1004

1 = PATIENT ID
2 = INSTITUTION (7,8, OR 9)
3 = -CATEGORY SURGERY
4 = -MONTH SCREEN
5 = -DAY SCREEN
6 = -AGE
7 = -SGPT VALUE
8 = -SGOT VALUE
9 = -ANESTHESIA
10 = -SUBTYPE TO PHA
11 = -NUMBER UNITS
12 = -MONTH REF. DATE
13 = -DAY REF. DATE
14 = -YEAR REF. DATE
15 = -STATUS IN STUDY
16 = DAYS FROM ENTRY
17 = ANTIGEN
18 = SGPT
19 = ANTI-HBS RIA
20 = ANTI-HBS PHA
21 = HOSPITAL-NOT HEP.
22 = HEPATITIS EXPOSURE
23 = HEALTH STATUS
24 = SX-NOT HEPATITIS
25 = ELIGIBILITY
26 = LENGTH-EPISODE HEP.
27 = HOSPITAL-HEPATITIS

GLOSSARY OF COMPUTER TERMS

VARIATE (OR VARIABLE)	NOTATION FOR A CHARACTERISTIC WITH MORE THAN ONE ATTRIBUTE. EACH ATTRIBUTE IS NUMBERED OR CODED.
RECORD	A SERIES OF VARIATES WHICH HAVE BEEN GROUPED FOR SOME LOGICAL REASON.
FILE	A HOLDING DEVICE FOR INFORMATION. A FILE MAY BE HUMAN READABLE OR COMPUTER READABLE. DATA MAY BE ALPHABETIC OR NUMERIC.
ASCII FILE	CONTAINS HUMAN READABLE INFORMATION. ALLOWS THE ENTRANCE AND RETRIEVAL OF HUMAN READABLE DATA TO AND FROM THE COMPUTER.
BINARY FILE	CONTAINS COMPUTER READABLE INFORMATION. EACH VARIATE IS TRANSLATED TO A PATTERN OF 0'S AND 1'S.
DIRECT ACCESS FILE	IN THIS TYPE OF FILE RECORDS MUST BE FIXED LENGTH AND MAY BE READ IN ANY ORDER.
SEQUENTIAL FILE	IN THIS TYPE OF FILE RECORDS MUST BE READ IN ORDER FROM THE BEGINNING OF THE FILE.
CURRENT FILE	THE FILE CURRENTLY BEING USED, WHICH IS A COPY OF A FILE IN PERMANENT STORAGE OR A FILE WHICH HAS NOT BEEN PLACED IN PERMANENT STORAGE.
PERMANENT FILE	A FILE WHICH HAS BEEN PLACED IN PERMANENT STORAGE. THIS MEANS THAT A COPY OF THIS FILE CAN BE RETRIEVED FOR USE. TO MODIFY A PERMANENT FILE CHANGES ARE MADE TO A COPY AND THIS NEW FILE REPLACES THE ORIGINAL FILE ENTIRELY.

LINE NUMBER	DESIGNATES WHERE DATA IS LOCATED IN AN ASCII FILE. THE FILE CAN BE MODIFIED BY ADDING OR DELETING SPECIFIC LINE NUMBERS.
DELIMITER	USED TO SEPARATE VARIATES IN AN ASCII FILE. A COMMA, A SPACE, OR A SERIES OF SPACES ARE THE ACCEPTABLE DELIMITERS.
ON LINE	THE TERMINAL IS CONNECTED TO THE COMPUTER BY TELEPHONE.
LOCAL	THE TERMINAL IS BEING USED TO PREPARE DATA FOR ENTRY INTO THE COMPUTER, BUT THERE IS NO CONTACT WITH THE COMPUTER AT THIS TIME.
RETURN KEY	THIS KEY ON THE COMPUTER KEYBOARD IS USED TO CARRIAGE RETURN. WHEN ON LINE, DATA ARE NOT TRANSMITTED TO THE COMPUTER UNTIL THE RETURN KEY HAS BEEN PUSHED, THUS ALLOWING FOR REVIEW AND CORRECTION.
PROGRAM	A DETAILED SET OF INSTRUCTIONS TO THE COMPUTER GOVERNING ENTRY, STORAGE, EDIT AND COMPUTATION OF DATA.

V. LABORATORY

V-A. MEASUREMENT OF ALANINE AMINOTRANSFERASE

PROCEDURES FOR USE OF THE
BECKMAN ENZYME ACTIVITY ANALYZER
SYSTEM TR

Revised 2/22/79

PREFACE

There have been wide differences in results of analyses of alanine aminotransferase (ALT) from laboratory to laboratory, even when the same method is used. Experts in clinical enzymology, both national and international, have been attempting to identify the factors involved in these variations in order to standardize the assay.

Reproducibility from laboratory to laboratory must be achieved in our Study to establish that differences among groups can truly be said to reflect differences in serum levels of the populations rather than differences among instruments or in the steps in the assay.

For practical purposes, there is no difference between 140 and 180 IU/1. In fact, in terms of our definitions, there is no difference between 60 and 70. There is, however, considerable difference between 41 and 49, and also between 85 and 95. We have not achieved that degree of precision (there is little point to discussing accuracy) from center to center. Furthermore, the upper limit of "normal" has been based on assays of sera from the control population, and variations <u>within</u> the normal range have an important influence on what we determine to be that limit. Finally, we are attempting to make a statement about the relationship of donor ALT levels to subsequent hepatitis in the recipient. The values we have called abnormal in the TTV Study group are much more tightly clustered in the range from 45 to 100 IU, and com-

parability again is a critical question in the reliability of our results and conclusions.

We have a better opportunity than most laboratories to achieve uniform results with ALT determinations. We all have the same instrument for the assay, which minimizes one variable. Nonetheless, each instrument differs from all others to at least some extent. If all other variables can be minimized in the comparison of a set of standards, the readings of the instruments can be made comparable by adjusting their individual calibration factors appropriately (above or below 1029).

A second advantage we have is the fact that at most centers the same technician does the testing most or all of the time. This should reduce our source of day-to-day intramural error.

The major problem for the group is intermural variation due to minor but potentially significant differences in operational habits. A meeting was held in September, 1976, for the technicians conducting tests in an attempt to achieve uniformity in every possible detail without regard to whether the particular way of doing each step could be the best.

This manual sets forth the detailed procedures upon which we agreed at the September meeting. Even though a particular detail seems unimportant, a uniform procedure for each item should take precedence. If there is a strong preference to the contrary, the individual should call it to the attention of the Coordinating Center or to the attention of the Group if the decision is considered arbitrary. Changes will be made from time to time, but they will be made only by the Group.

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COMPLETING THE DAY'S ACTIVITIES

SUPPLIES NEEDED FOR ALT TESTING

Name	Use and/or Source	Storage Instructions
'ater	For cleaning air filter located on underside of the instrument.	Not applicable
Deionized water or deionized-distilled water	For wash bottles. Derived from the usual supply for the laboratory.	Not applicable
Sterile water	For reconstitution of lyophilized reagent (Enzymatic ALT Reagent). Sold by Abbott Labs.	After seal is broken store at 4°C.
)ne polyethylene bottle (6 oz.) labeled "Wash Line Water"	For deionized-distilled water to be used in wash pickup line.	Not applicable
)ne polyethylene bottle (4 oz.) labeled "Enzymatic ALT Reagent Line Wash Water"	For deionized-distilled water to be used in the Enzymatic ALT Reagent wash pickup line.	Not applicable
)ne polyethylene bottle (4 oz.) labeled "Sterile Water"	For sterile water to be used in reconstituting Enzymatic ALT Reagent.	Not applicable
ink or bottle (32 or 4 oz.) for draining. f bottle is used, label TR Drain"	To contain ejected wash water, used reagent, and assayed serum.	Empty frequently following rules of laboratory for disposal of potentially infectious wastes.
ne-nosed pliers or imilar instrument e.g., hemostat)	To remove aluminum cap from the Enzymatic ALT Reagent bottle.	Not applicable
0 ml sterile disposable ipettes	For delivery of 15.5 ml of sterile water in reconstituting Enzymatic ALT Reagent. Sold by various suppliers.	Store to safeguard sterility
spirator bulb	For pipetting sterile water in reconstitution of reagent.	Not applicable
nall test tube	To hold 2 ml of sterile water for filling sample cup in "0" position.	Not applicable
asteur pipette	For transfer of sterile water from test tube to sample cup in "0" position.	Not applicable
imwipes or other lint-ee tissues	For cleaning probe and wiping external surface to remove dust and dirt. Purchase from supplier.	Not applicable
obe cleaning wires	To remove any dried-out protein from the barrel of the probe. Supplied by Beckman.	Not applicable

SUPPLIES NEEDED FOR ALT TESTING (CONT.)

	Use and/or Source	Storage Instructions
yringe with line of lastic tubing	For removing water when changing cuvette. Supplied by Beckman.	Not applicable
LT Control I vials	To serve as a standard to check operation of instrument and integrity of reagents. Should be in general range of upper limit of normal. Supplied by Coordinating Center.	Store at or below -70°C
LT Control II vials	Same type of standard as ALT Control I, but level should be near that used to define an episode as hepatitis.	Store at or below -70°C
50-microliter plastic ups	To hold aliquots of controls and samples to be tested. Purchase from Beckman.	Not applicable
utomatic 150 microliter ipette and disposable ips	For transferring 150 microliters of sample to sample cups	Not applicable
LT reagent (Enzymatic LT Reagent)	Purchase from Beckman.	Store lyophilized at 4°C
m for stopping turn- le	If tray is not fully loaded, the arm stops turntable after last sample has been tested. Supplied by Beckman.	Not applicable
mall, round-bottomed est tube (e.g., 10 x 5 mm)	When reagent in manufacturer's bottle is nearly exhausted, transfer of residual to test tube will insure maximal utilization without risk of picking up incomplete aliquot. Purchase from any supplier.	Not applicable
asteur pipette with ubber bulb	For making transfer of residual Enzymatic ALT Reagent (for last few tests) from manufacturer's bottle to small test tube.	Not applicable
ydrogen peroxide, 3% olution	For treatment of the internal lines of instrument to prevent microbial contamination.	Capped and at room temperature. Should not be used for more than one to two weeks after opened because solution deteriorates.
odium hypochlorite	For inactivation in the drain of any virus derived from the sera tested. May be purchased at any grocery store as Clorox.	Store tightly capped

LOCATION OF THE INSTRUMENT

The SYSTEM TR should be kept on a level surface in an area where external conditions in the room will not interfere with its internal environment. It should be on a table or bench that is as free of vibration as possible. Dust or smoke in the air may drift into the interior of the instrument and its light path, causing erratic and possibly erroneous readings. The instrument can most efficiently bring the reactants to 37°C (99°F) if the usual room temperature is approximately 20°C to 22°C (68° to 72°F). It should not be used where there is substantial deviation from this range. The temperature in the immediate vicinity of the instrument should not differ from the room temperature. Accordingly, it should not be placed next to the opening of a forced air duct from an air conditioner or heating system.

The SYSTEM TR has circuitry intended to minimize fluctuations in line voltage. These equalizers, however, are capable of buffering the instrument only to a certain extent. If it is on the same power line with other instruments, especially those that are turned on and off frequently, erratic results may be obtained. At certain hours of the day, results may be consistently good or poor. If this happens, the instrument user may find it advantageous to rearrange his or her schedule accordingly. If the situation is very bad, the possibility of a separately wired outlet should be discussed with the investigator in charge of the center. Other instruments and motors in the same or adjacent rooms may adversely affect the

SYSTEM TR not only by their varying demand upon the electrical supply, but also by the electromagnetic fields they create. Fluorescent lights in the room, and even in adjacent rooms (especially that immediately beneath the instrument), should not be turned on or off during a run.

If poor reproducibility is a problem, all of these factors must also be considered. Relocation of the SYSTEM TR or of the other equipment may be the only answer.

After installation, the instrument should always be kept on. In that way, it is not only always ready for use, but also "wear and tear" on the electrical components is minimized. If it has been turned off, one to two hours should be allowed for the circuitry to stabilize before proceeding with the pre-run preparation of the instrument.

PREPARATION FOR A RUN

Before the operator can prime the instrument with reagent and set the controls, the following must be done: (1) The instrument must be primed with fresh deionized-distilled water or deionized water, the filter and sample probe cleaned, and its functioning checked, (2) the Enzymatic ALT Reagent must be reconstituted and (3) one vial each of the ALT Controls I and II must thaw. Although these activities are described separately, they are usually carried out within the same time period (approximately 15 minutes).

INSTRUMENT PREPARATIONS

WASH, ENZYMATIC ALT REAGENT, AND DRAIN LINES. At the beginning of a day's operations, the two pickup lines will ordinarily be resting in their respective wash bottles on the shelf of the front cavity. The plastic line to the operator's left is the pickup line for wash water (WASH LINE) and the plastic line on the right is the pickup line for Enzymatic ALT Reagent (ENZYMATIC ALT REAGENT LINE). The two bottles should be labeled "WASH LINE WATER" and ENZYMATIC ALT REAGENT LINE WASH WATER." In the list of needed supplies, we ask the operators to use two different sizes of bottles (6 oz. and 4 oz., respectively) to minimize the chance of inadvertent reversal. The operator should remove the pickup lines from the bottles. Each bottle should be filled with a fresh supply of the deionized or deionized-distilled water that is usually used in the laboratory. The sterile water supplied for Enzymatic ALT Reagent reconstitution should not be used for the wash lines -- that quality of water is not needed for this purpose, and the cost of its use would be prohibitive. The bottles should be placed under their respective (and therefore uncrossed) pickup lines. This will prevent contamination of the wash line water with residual reagent on the outside of the Enzymatic ALT Reagent line.

The yellow drain line extending from the bottom left of the instrument can be placed in a sink if the sink is below the level of the instrument.

If such a sink is not available, the line can be placed in a 32 to 64-oz. container labelled "TR Drain" containing 20 ml per liter of expected drainage of 5% sodium hypochlorite solution (Clorox). The material coming from the drain during a run is potentially infectious (from the sera being tested) and should be handled according to the laboratory rules for disposing of contaminated waste. In addition, care should be taken to avoid microbial growth in the drain bottle by emptying and washing the bottle daily. Five ml of sodium hypochlorite, 5% is then added.

WASH CYCLE. With the wash bottles in place, the operator should depress the PRIME BUTTON. Wash water will be pumped into both parts of the system, thereby flushing the instrument with fresh wash water. Each wash cycle takes slightly over 2 minutes. When it is completed, the START and PRIME buttons automatically light. The operator should then push the PRIME button again to begin a new cycle. This should be repeated three times, for a total of four washes. While a cycle is in operation, the following activities should be conducted.

CLEANING OF THE FILTER. The filter support which is on the underside of the instrument should be pulled straight out and removed from the instrument. If needed, the filter should then be removed from the support and cleaned by running tap water over it. Excess moisture should be removed by shaking, and the filter and its support reinstalled in the instrument.

CLEANING THE SAMPLE PROBE. To gain access to the sample probe for cleaning, the sample turntable and the front cover of the instrument should be removed. Next, the crane switch should be turned to OFF, and the crane itself swung counterclockwise through the door of the instrument. This makes the probe accessible for handling. The outside of the probe should be wiped with a dry Kimwipe or other lint-free tissue, and the cleaning wires inserted into the probe several times to remove any dried-out protein from previous runs.

POSITIONING THE PROBE. When the crane is swung back inside the instrument, the vertical clearance of the probe above the edge of the cuvette cover flange should be 1/16th inch. If adjustment is necessary, the screw at the attachment of the probe should be loosened with one's fingers. The probe can then be manually raised or lowered. If the probe is too high, it may pick up only a partial aliquot of serum, resulting in an erroneously low reading. If it is too low, it may strike the flange or the bottom of the sample cup, causing it to miss the next cup when it swings out to pick up that sample. The crane switch should now be turned on and the turntable returned to position.

CHECK OF SYRINGES. While the front cover is off, a check should be made of the operation of the four engaged syringes that function when an air "blank" or "reference" is being used. The pistons of the front syringe of the two in the "substrate pump assembly," the front two syringes of the four in the "drain pump assembly," and the left syringe of the two in the "sample pump assembly" should all move

smoothly up and down. These assemblies are shown in section six, page 6-1, of the Beckman Operating and Maintenance Manual. Observe carefully to see that the green tip of each piston moves up and down with the arm of the piston; otherwise no material is pumped. The front cover should then be replaced.

ENZYMATIC ALT REAGENT PREPARATIONS

To determine the amount of Enzymatic ALT Reagent needed for the run, the number of samples to be tested should be counted. One 15.5-ml bottle of ALT reagent will be needed for each 20 samples. That number of bottles of lyophilized Enzymatic ALT Reagent should be removed from storage at 4°C for reconstitution.

OPENING OF THE BOTTLE. The Enzymatic ALT Reagent should never be reconstituted by adding water to the bottle through the rubber diaphragm with a sysringe and needle. The excessive agitation can cause partial destruction of some constituents, especially NADH and LDH. In addition, air bubbles are trapped for prolonged periods, causing measurement of volume to be inaccurate and interfering with the transmission of light when the cuvette is filled.

FIRST, the aluminum seal should be removed from the top of the Enzymatic ALT Reagent bottle. It is suggested that fine-nosed pliers or a similar instrumen

a hemostat is usually satisfactory - be used to lift up and tear off the seal. If the fingers are used, the often jagged edges can cause cuts. Next, a thumbnail should be placed between the lip of the glass and the rubber stopper and the stopper slowly pushed to break the vacuum in the bottle as gently as possible. Hasty or gross handling must be avoided while removing the stopper, because a sudden rush of air into the bottle will cause powder to spray all over the laboratory. The concentration of some or all of the component reagents will then be low, and an optimum ALT reaction may not be achieved. On the other hand, if there is no evidence of a vacuum, that bottle should be discarded and another bottle of Enzymatic ALT Reagent used. (In the presence of air and moisture, the chemicals deteriorate.) When removed, the rubber stopper will also have powdered agent adhering to it, and should be placed upside down on a laboratory bench to conserve this material. Before the water is added, the Enzymatic ALT Reagent bottle should be tapped gently to knock the powder that gathers in its neck into the bottle itself.

MEASUREMENT OF WATER FOR RECONSTITUTION. To avoid one of the many possible variables from laboratory to laboratory, the sterile water sold by Abbott Laboratories will be used for reconstituting the Enzymatic ALT Reagent. This is labeled "Sterile Water" and is of a quality suitable for intravenous administration to humans. It is supplied in 250-ml bottles. Once a bottle is opened, the date it was opened should be marked on the label, and the bottle stored at 4 degrees Centigrade with the cap tightly closed.

The volume of sterile water needed for the day should be carefully poured into the 4 oz. bottle labelled "STERILE WATER" and the stock 250-ml bottle returned to 4°C storage.

A sterile 10 ml disposable pipette with an aspirator bulb (Propipette or equivalent) should be introduced into the aliquot of sterile water and filled to the "0" mark. Two ml should be delivered into a small test tube for later use (in testing the zero rate reaction). Seven ml should then be carefully added to the Enzymatic ALT Reagent. As the water is delivered, the bottle should be turned so that all the reagent is washed from the neck of the bottle. This process contaminates the top of the pipette, if care is taken to wash the reagent adherent to the neck of the bottle. A second sterile pipette should then be introduced into the sterile water, filled to the "0" mark, and 8.5 ml delivered into the Enzymatic ALT Reagent bottle. A total of 15.5 ml has now been added.

GETTING THE LYOPHILIZED REAGENT INTO SOLUTION. Once water has been added to the reagent, the rubber stopper should be carefully lifted off the laboratory bench and replaced in the bottle. Then, the bottle should be rolled gently several times until the lumps in the powder begin to disappear. The bottle should never be shaken or handled roughly because both denaturation of reagent and trapping

of air will occur. The capped Enzymatic ALT Reagent bottle should then be kept at room temperature for at least 15 minutes so that all the material goes into solution. During this time, the bottle should be rolled frequently. The fact that solution of reagent is complete can be discerned from the appearance of the upper side of the bottle, when held in a horizontal position. If not in solution, the inside surface will be slightly opaque and/or granular; if solution is complete, it will be clear. As soon as solution is complete, the bottle should be stored at 4°C until needed that day. Do not use reagent prepared the day before or earlier.

CONTROL PREPARATION

While the wash cycles are being repeated and the reagent is going into solution, the controls can be thawed. One vial each of ALT Control I and ALT Control II should be removed from storage. The vials should be placed on the laboratory bench at room temperature until the two control sera have thawed. Thawing may be hastened by holding the vials in one's hands. After thawing, the operator should gently invert the vial three times to be certain that cryoprecipitated proteins return to solution.

SAMPLE PREPARATION

SAMPLE PREPARATION. Small plastic cups of 250-µl capacity are used for testing the samples. They are placed in the tray of

the turntable. The tray is marked with the numbers "0" to "19" to identify the samples. The "0" position is always reserved for an aliquot of sterile water, while the 1 and 2 positions are reserved, respectively, for aliquots of Control I and Control II. These controls are always tested first to determine whether the instrument is functioning properly. For the two control samples, the usual values at each particular laboratory will be determined through repeated testing over a period of time. In addition, two more aliquots of each control should be used in each tray. They are placed in middle and end positions (as described below). Accordingly, a full tray (13 patient or donor samples) will have the following pattern: an aliquot of sterile water in position "0," Controls I and II in positions "1" and "2," "10" and "11," and "18" and "19," respectively. The patient samples will be in all other positions.

If only one patient sample is to be tested on a given day, the operator should still run three aliquots of each of the two control samples, plus the aliquot of sterile water in the "0" position. In such a case (presumably infrequent) the first pair of controls would occupy positions "1" and "2;" the second pair, positions "3" and "4;" the sample, position "5;" and the third pair of controls, position "6" and "7." For numbers of sera intermediate between one and thirteen, one-half should be placed between the first and second pairs of controls, and one-half between the second

and third pairs. If the number is odd (e.g., nine specimens), place the smaller number (i.e., four specimens) between the first and second pairs of controls and the larger number (i.e., five specimens) between the second and third pairs.

When the number of samples to be analyzed only partially fills the tray, the operator should insert the small plastic arm in the first empty position in such a way that it will trigger the "stop mechanism" after the last sample is tested.

On the ALT RUN FORM (see following page), the operator should identify each control or sample by its position on the tray, the designation of the control or name and Study number of the patient, and the sample cups should then be marked with the position number and lined up in front of respective samples. Before transferring the sample, however, each cup should be checked for cracks and for extraneous material such as dirt, ashes, and hair.

TTV STUDY ALT RUN FORM

Test date: _____

Calibration number: _____

EAR Lot No. _____ EAR absorbance: _____

	Tray position number	Sample Identification	Date drawn	
	0			
	1			ATTACH
	2			PRINTOUT
	3			TAPE
	4			HERE
	5			
	6			
	7			
	8			
	9			
	10			
	11			
	12			
	13			
	14			
	15			
	16			
	17			
	18			
	19			

The cup in the "0" position should be filled with the sterile water set aside previously when the Enzymatic ALT Reagent was being reconstituted. A Pasteur pipette may be used for this purpose.

The controls and samples, however, should be transferred to the cups with a 150-µl automatic pipette. A new tip should be placed on the pipette for every sample taken.

The size (250 µl) of the cup to be used and the size of the sample aliquot were standardized because differing surface area-to-volume ratios result in different rates of evaporation. Standardization will not eliminate all of the evaporation, but hopefully will make it comparable from center to center. The loaded tray should be covered immediately with the tray cover and kept covered at all times. In addition, the cups should be filled no more than 15 minutes before the run is actually started.

PRIMING THE INSTRUMENT WITH REAGENT

While the samples are being prepared, the instrument should be primed with Enzymatic ALT Reagent. One does this by removing the Enzymatic ALT Reagent pickup line from the bottle of water labeled ENZYMATIC ALT REAGENT LINE WASH WATER" and placing the plastic line in the bottle of reagent. The WASH LINE WATER bottle is left in place but should be checked

to be sure it is almost full. If it is not, it should be refilled with the deionized or deionized-distilled water. The operator should then push the PRIME button to purge the wash water from the Enzymatic ALT Reagent system and replace it with reagent. When this cycle is completed, the START and PRIME buttons will become illuminated.

When the reagent enters the Enzymatic ALT Reagent line it is heated to and held at 40°C (to result in a final temperature of 37°C when mixed with the specimen in the cuvette). If the interval between priming and the run is more than 10 minutes, the instrument should be reprimed to flush out the materials subjected to that elevated temperature.

SETTING OF INSTRUMENT CONTROLS

After the sample cups are in position on the turntable tray, the tray should be returned to the instrument, with the turntable switch in the OFF position and the tray cover in place.

The operator should first press the SGPT (=ALT) button in the vertical set on the left side of the instrument to denote the enzyme to be determined. To gain access to the other instrument controls, the cover to the upper right of the instrument should be

swung down. The controls should then be adjusted to the following values, in the sequence given.

Control	Value
0% T	.000 ± .002
100% T	100.0 ± 0.2
ABS	.000 ± .002
TEMP(°C)	37.0 ± 0.1
ZERO RATE	0 ± 3
ABS OF REAGENT	.800 or higher
CAL	1029
STERILE WATER TEST (ZERO RATE CHECK)	0 ± 3

First, the "0%T" button should be pushed to establish that there has been no "drift" in the circuitry. The digital display will change and the numbers approach .000. When the instrument has "found" its adjustment, the calibration button (labelled "CAL") will light. Unless the reading actually is .000 ± .002, the setting should be manually adjusted. The knob should be turned until the numbers displayed are within the limit given. Clockwise turning will increase the numbers, while counterclockwise adjustments will decrease the numbers. Note that the CAL light goes off as the adjustment knob is turned and relights after the adjustment has been made and the circuitry restabilizes. This process may need to be repeated several times before the reading is within the limits indicated. When the proper setting is achieved, the lock may be set to

the operator's own touch-i.e., it should be tight but not unduly difficult to turn.

The 100%T button should be pushed. The procedure for adjustment of the reading to 100.0 ± 0.2 is the same as described above.

One hundred percent transmission through the water-filled cuvette is equivalent (by definition) to no absorption of light. To determine whether the circuitry is reading transmission and absorption equivalently, push the button labelled "ABS." The digital display should then change to .000 ± .002 (100%T is the same as zero absorbance). If not, the 0%T and 100%T settings should be rechecked and the necessary adjustments made.

The next setting to be verified is the cuvette temperature, which must be at 37.0° ± 0.1°C. The operator presses the button labelled "TEMP" to check the display. If the temperature reading is incorrect, the Beckman serviceman should be called and the run postponed until the temperature is correctly set.

The instrument must be "Zero Rated," or adjusted to zero rate activity. To accomplish this, push the START button and then the ZERO RATE button. The probe will swing out, pick up 50 µl of sterile water from the "0" position on the tray and deliver 35 µl

to the cuvette which now contains Enzymatic ALT Reagent. The chemistry indicator--a rectangular block on the instrument panel--will light up. The operator should wait for the CAL light to go on, then read the display, and turn the knob on the ZERO RATE button to dial in zero.

The absorbance of the reagent is next checked by depressing the ABS button. The display value should be .800 or higher. If it is not, the NADH may have deteriorated in the Enzymatic ALT Reagent and should not be used.

If the reading is .799 or less, a new bottle of Enzymatic ALT Reagent with a satisfactory absorbance (i.e., concentration of NADH) must be found. If this is necessary, the controls and serum samples in the cups must be discarded. Even if the cups are covered with plastic and placed in a refrigerator, evaporation and other changes in the sample make any subsequent results questionable.

A printout of the reagent absorbance should be obtained for the ALT Run Form by pushing the PRINT button located on the special function switch board.* The absorbance check is ended by pressing the START button to terminate the chemistry cycle so that the next

*A printout of any value can, of course, also be obtained by pushing the print button. However, the reagent absorbance is the only control value needed for the Run Form.

step can be taken. The reagent absorbance value should be recorded on the Run Form.

The primary calibration number should always be verified. The operator should press the CAL button and read the display. If 1029 is not displayed, the adjustment knob should be unlocked and the primary calibration number dialed in until a value of 1029 is reached. Be sure to lock the adjustment knob when finished.

The final check is made of the ZERO RATE adjustment by testing the sterile water in the "0" tray position. The START button should be pressed and the printed value should be 0 ± 3 IU/1. If the value is not within these limits, then the ZERO RATE should be readjusted and the water tested again. The calibration number should always be verified when the ZERO RATE has been adjusted.

THE DAY'S FIRST RUN

The automatic run can now be started. The tray cover should be removed, the turntable moved clockwise to position "1," and the turntable switch placed in the ON position. The tray cover should be put back on and a check made to see that the sample cups

are so positioned that their tops are all just below the level of the cover. If all is in readiness, the START button should be pressed to begin the automatic run. The probe will automatically pick up the successive samples for testing.

The results of each test will be automatically recorded by the printer attached to the instrument.

After the values for Control I and Control II (positions "1" and "2") have been printed, they should be checked to ensure that they are within limits acceptable to the laboratory (see "Evaluation of the Validity of Each Run," page V-27). If they are not, the entire series of checks should be repeated.

It will take approximately 40 minutes for one full tray of samples to reach the last position. The operator, however, should check the status of the run after 30 minutes, making sure that the wash line bottle has plenty of water and that the Enzymatic ALT Reagent pickup line is well below the surface of the liquid. The operator may find that the amount of Enzymatic ALT Reagent is sufficient for the remaining samples but its shallowness in the bottle makes questionable the pickup of a complete aliquot. To avoid using a new bottle of Enzymatic ALT Reagent for a few tests, carefully transfer the Enzymatic ALT Reagent pick-up line to a small round-bottomed test tube. Using a Pasteur pipette with bulb, transfer the remaining reagent from the bottle to the tube.

After the run is completed, turn the turntable switch to OFF, remove the turntable, and dispose of the cups into a receptacle suitable for infectious wastes. Tear off the printed tape and paste it on the ALT RUN FORM.

ADDITIONAL RUNS ON THE SAME DAY

For additional runs on the same day, the steps identified above are repeated, but with some variations.

The Enzymatic ALT Reagent pick-up line is transferred to a second bottle of Enzymatic ALT Reagent, which was reconstituted before the first run and has been kept at 4°C to this point. With a Pasteur pipette, the remaining reagent in the first bottle should be added to the second bottle. This can be done, however, only if the reagents in the two bottles are from the same lot. If the bottles are marked with different lot numbers, the residual in the first bottle should be discarded, and the instrument flushed with the new lot of Enzymatic ALT Reagent by pushing the button for the PRIME cycle after the controls and samples are prepared.

The level of water in the wash bottle should be checked, because most of it may have been used in analysis of the first tray. Refill the bottle, if needed, with deionized-distilled water.

The instrument controls (0%T, 100%T, ABS, TEMP, ZERO RATE, ABS, CAL) should be re-set in the same sequence as for the first run. In most instances some readjustment will be necessary.

EVALUATION OF THE VALIDITY OF EACH RUN

The TR operator is the person responsible for the technical reliability of all ALT results reported. Before giving the results from a run* to any other person connected with TTVS, he/she should determine the validity of the values in that run. The run is defined as *valid* if, and only if,

1) The Study protocol "Procedures for Use of the Beckman Enzyme Activity Analyzer System TR" was followed exactly.

2) The Beckman system TR performed to specifications (i.e., there was no question of malfunction of the instrument during the run). Examples of problems in this category would include:

   a) Zero rate was unstable.

   b) There was no reduction in substrate or wash water volume.

   c) Stirrer was not moving (i.e., made no sound).

   d) Printout showed Δ, R, or T repeatedly.

3) The three individual determinations for Control I and the three for Control II have a coefficient of variation** not exceeding ten percent.

4) The mean of the three Control I and three Control II determinations in the run fall within the range of plus or minus two standard deviations of the mean currently being used for the individual institution.

*One trayload (whether full or partly full) of samples and controls.

**See "Calculations for ALT Control I and Control II" on page V-27

If the run is accepted as valid, the ALT values for Control I and Control II are recorded on Form 8, and those for the patients and donors are released to the person(s) designated to receive them.

If one or more of the four criteria for a valid run is not met, the run is invalid, and the ALT values are not reported. The operator's supervisor is notified and the "Out of Control" procedure is initiated.

Calculations for ALT Control I and Control II

1. Coefficient of variation for one run

		Control I	Control II
ALT Value	Beginning=		
	Middle=		
	End=		
Mean	(sum÷3)=		
Range	(largest-smallest)=		
Standard Deviation	(range x 0.6)=		
Coefficient of Variation	($\frac{S.D.}{mean}$ x 100)=		

2. Quality control ranges for Control I and for Control II

At the end of each calendar quarter, the Coordinating Center will promptly re-calculate for the individual institutions the mean and standard deviation of reported determinations. This re-calculation will take into account ALT levels reported during that period on Form 8 for Control I and Control II. Those values, to be used during the ensuing quarter, will be supplied by computer message addressed to the TR operator.

"Out of Control" Procedures

1. Review the protocol of the procedure for potential sources of error.

   a. The wrong reagent(s) were used (sterile water, substrate, wash water).
   b. There was a mistake in volume measurements.
   c. Glassware and reagents were free of possible contamination.
   d. Setting of instrument was incorrect (wrong button in for the procedure, wrong calibration number, transmission settings, zero rate).
   e. Instrument has not been properly maintained.
   f. Improper handling or storage of Controls.

2. If the Beckman System TR has a malfunction that is obvious during the run, the run should be stopped. A problem may not be appreciated, however, until results for the Controls are seen to be deviant. If review of the procedure

discloses no problem, it is possible that the machine is at fault. Check the "troubleshooting" section of the TR Manual (page 6-7).

3. If no difficulty is found, repeat testing in triplicate of Control I and Control II, using a new vial of each, and also freshly prepared reagents.

4. If the results of the rerun are still deviant, call Beckman "hot line" or Coordinating Center.

5. Once the problem has been corrected, as evidenced by mean values for both Controls within acceptable limits, the patient and donor specimens are reassayed.

COMPLETING THE DAY'S ACTIVITIES

The reagent bottle or test tube should be removed from the reagent line and discarded. The outside of the reagent pick-up line should be wiped with a dry Kimwipe or equivalent tissue and placed in the wash bottle labelled for the reagent pick-up line. The PRIME button should be pushed to begin a new wash cycle, and the cycle should be repeated twice. The drain bottle, if used, should be emptied and cleaned as described earlier. The instrument should be left on. The sterile water bottle should be emptied and stored dry.

V-B. ALT CONTROLS FOR THE BECKMAN TR

Reproducibility of readings of Alanine Aminotransferase activity from laboratory to laboratory must be achieved in the TTV Study to establish that differences among groups can truly be said to reflect differences in serum levels of the populations.

In order to eliminate variations in results of ALT analyses due to differences in steps in the assay, the Study group prepared a detailed and exacting protocol for the use of the Beckman Enzyme Activity Analyzer System TR (Section V-A of this manual).

As a check on daily instrument function within each laboratory, two controls were prepared by the coordinating center. The purpose of Control I was to check accuracy and comparability of readings in the borderline range of abnormal ALT activity, while the purpose of Control II was to similarly check results in the definitely abnormal ranges. The daily accuracy of individual Beckman instruments is monitored by the placing of aliquots of each control at the beginning, middle, and end of each set of sample runs. The

coordinating center also uses the results of these control readings to verify comparability between centers.

This section consists of the current ALT Controls in use by all centers.

TRANSFUSION-TRANSMITTED VIRUSES
A Cooperative Study

COORDINATING CENTER
USC SCHOOL OF MEDICINE

Liver Service/1200 Lab Bldg.
Rancho Los Amigos Hospital
7705 Golondrinas Street
Downey, CA 90242

TELEPHONE
AREA CODE 213
740-5111

November 26, 1979

To: Beckman TR Operators and Laboratory Supervisors

From: Coordinating Center

Subject: New ALT Controls for the Beckman TR

The accompanying shipment contains TTV-ALT Control I, 1980, and TTV-ALT Control II, 1980. Each center has been shipped 20 boxes of 36 vials of Control I and 20 boxes of 36 vials of Control II, for a total of 720 vials of each.

The controls should be stored at -70°C or lower immediately upon receipt. Please call Virginia Edwards at the Coordinating Center to verify arrival of the shipment (213-922-7231).

As of December 1, 1979, the ALT controls currently in use should be replaced by these new controls. The new controls should not be used before that time.

Thawing and testing will continue as described in the TTVSG manual, "Procedures for Use of the Beckman Enzyme Activity Analyzer System TR," revised April 1979. The control vials contain 600 microliters of frozen sera, which is ample for the three aliquots of 150 microliters required for each turntable tray of TTV study samples tested. The ALT value of each control will be determined by the individual center. Use the enclosed revised Form 8, ALT Controls for Beckman TR, to record the ALT values obtained. Continue to mail the completed Form 8 to the Coordinating Center weekly.

The pooled human sera used to make the controls may contain hepatitis viruses B and non-A, non-B, so use appropriate caution in handling and disposal.

Please replace pages V-33 and V-34 in your TTVSG Operations Manual with the enclosed copy.

VE:dm
encl

cc: Drs. Aach, Hollinger, Mosley, Stevens, Szmuness, Weiner

TTV-ALT CONTROL-I 1980

TTV-ALT CONTROL-II 1980

CENTER ______________

TTV-ALT Control No.	Date of Test YYMMDD	Enzymatic ALT Reagent (EAR) Lot No.	Storage Temp °C	ALT Values (I.U.) 1st Obsv.	2nd Obsv.	3rd Obsv.	Mean	C.V.	

Revised November 1979

V-C. ALT QUALITY CONTROL PANEL

Previous analyses by the Transfusion Transmitted Viruses Study group of samples of known value with the Beckman TR had not been definitive in identifying problems of measurement accuracy and reproducibility. The Study group therefore inaugurated a blinded study of Beckman TR instrument variation through time in the measurement of Alanine Aminotransferase (ALT).

The ALT Quality Control Panel is a series of blinded samples used in the measurement of ALT with the Beckman TR instrument. Neither the laboratory personnel nor the clinical investigators are privy to the code break. This is maintained independently by the data management group located at the coordinating center. The reference laboratory in the coordinating center participates in the study as a contributor; as such, this laboratory also is blinded.

Consecutively numbered samples are sent to each center. The number is preceded by a digit which is the center identification number. The samples consist of a randomized set of ALT values for each center, so sample 001 in one

panel is not necessarily the same as sample 001 in another panel. The samples included enable consideration of values of ALT well within the normal range as well as values in the range considered to be indicative of hepatitis. The random presentation of these standards in a blinded fashion offers the opportunity to test instrument variation through time rather than a combination of human and instrument sources of error. Removal of the "expected value" reduces opportunities to adjust the procedure in order to obtain desired values. This feature, coupled with the detailed protocol for specimen preparation and equipment maintenance described in Section I of this manual, should reduce all non-instrument variation.

This section consists of the current procedures regarding the use of the ALT Quality Control Panel with the Beckman TR.

TRANSFUSION-TRANSMITTED VIRUSES
A Cooperative Study

COORDINATING CENTER:
USC SCHOOL OF MEDICINE
2828 SOUTH HOPE STREET
LOS ANGELES, CALIFORNIA 90007

TELEPHONE
AREA CODE 213
748-3111

MAY 22 1978

TO: Participating Centers

FROM: Coordinating Center

SUBJECT: ALT Quality Control Panel Two

The ALT Quality Control Panel Two is composed of 100 samples of 1 ml each, labeled consecutively from 001 to 100. The number is preceded by a digit which is the center identification number. For example, St. Louis will have samples 1001 through 1100, and Baylor will have samples 2001 through 2100. The panel is a randomized set of ALT values for each center, so sample 001 in one panel is not necessarily the same as sample 001 in another panel.

The purpose of testing with the ALT Panel is to verify that the four Beckman TR instruments used in the TTV study will produce comparable results, as they must if we are to pool the patient data. The following protocol is the best mechanism by which to determine whether these instruments provide usable data through time.

Please read the ALT Panel protocol carefully. If any requirement cannot be met by your center, notify the Coordinating Center immediately. The problem will be resolved so that all three centers and the Coordinating Center will be following identical protocols.

Please follow the protocol exactly, as the only permissable variable is the instrument. If an error should occur, please notify the Coordinating Center immediately. It should be recognized that the purpose of this study is not to describe the Beckman TR under <u>usual</u> conditions, but under very <u>precise</u> conditions.

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Encl.

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PROTOCOL: ALT QUALITY CONTROL PANEL TWO

I. STORAGE OF ALT PANEL TWO

When the ALT Panel arrives packed in dry ice, transfer immediately to a -70°C or lower freezer. Any temperature above -70°C is not acceptable, as the storage temperature must be consistent in all centers. Check the temperature of the freezer prior to each test, correct any deviation, and notify the Coordinating Center of any change in temperature.

II. WHEN TO TEST PANEL TWO

One sample from the ALT Panel is to be tested only when there is a full tray of TTV Study samples to test for ALT. This requires 13 TTV study samples, one TTV-ALT Control-I/1978 (3 replicate tests), and the panel sample (3 replicate tests), making a total of 19 tests and a full tray on the Beckman TR turntable.

The TTV-ALT Control-II/1978 is not to be tested with this panel full-tray run. Our concern, considering the TTV study patient population, is with performance of the Beckman TR in the lower ALT range, so the lower level ALT control was selected.

If there are fewer than 13 study samples to be run, do not include a sample from the panel. The reason for a full tray load is to control the time interval between the first, second, and third observation of the panel sample replicate tests.

Test one sample at a time from the ALT panel following the instructions under Method. The panel must be tested in numerical order (i.e., test sample #001 first, #002 second, etc.), each with a full-tray load.

If there are enough TTV study samples (13) to run a second full tray on a given day, include the next sample from the ALT panel.

III. METHOD TO TEST ALT QUALITY CONTROL PANEL TWO

A. Determine whether there is a full tray (13) of TTV study samples as described above. Record the time the method was initiated (Step B begun) on Form X. The total time to complete Step B through Step I should not exceed two hours.

B. Reconstitute Beckman Enzymatic ALT reagent with 15.5 ml of sterile water as described in "Procedures for Use of the Beckman Enzyme Activity Analyzer System TR," revised 2/22/79. Record lot number and expiration date of reagent on Form X.

C. Pull from freezer one aliquot of the TTV-ALT Control-1/1978 and allow the sample to thaw at room temperature. Mix by inverting gently 3 times and transfer 150 µl to three labeled sample (250 µl) cups. Record necessary information on Form X.

D. Pull from -70°C or lower freezer ALT Quality Control Panel Two Sample #001 (if already tested, pull #002, etc.). Allow sample to thaw at room temperature. Mix by inverting gently 3 times, and transfer 150 µl to three labeled sample (250 µl) cups. Record sample number and storage temperature on Form X.

E. Prepare TTV Study Samples and transfer 150 µl to sample cups.

F. Load turntable tray with sample cups as follows:

Position 0	Sterile water for Zero Rate and Absorbance
Position 1	ALT Control-I
Position 2	ALT Panel Sample #001 (or #002, etc.)
Position 3-9	TTV Study Samples
Position 10	ALT Control-I replicate
Position 11	ALT Panel Sample replicate
Position 12-17	TTV Study Samples
Position 18	ALT Control-I replicate
Position 19	ALT Panel Sample replicate

G. Follow "Procedures for Use of the Beckman Enzyme Activity Analyzer System TR," as described in the 2/22/79 revision, to initiate the run.

H. Upon completion of the 19th test position, record the time terminated on Form X.

I. Record the ALT values for the Control and the Panel sample from the printout tape on Form X.

IV. MACHINE FAILURE OR TECHNICAL ERROR DURING ALT QUALITY CONTROL PANEL TEST

If for any reason the above protocol is interrupted during the panel test, complete Form X to the interruption point and describe failure under comments.

V. FORM X — ALT QUALITY CONTROL PANEL TWO

After each panel sample is tested and Form X completed, send the form to the Coordinating Center. We will expect to receive several completed Form X's each week from all centers.

FORM X - ALT QUALITY CONTROL PANEL TWO — TTV STUDY 1978

CENTER _____

BECKMAN TR OPERATOR _____

DATE OF TEST _____

BECKMAN ENZYMATIC ALT REAGENT

Lot No. _____

Expiration Date _____

Reagent Absorbance _____

TIME (Record A.M. or P.M.)

Method Initiated _____

Automatic Run Initiated _____

Automatic Run Terminated _____

V-ALT

CONTROL-1

Control date _____

Storage temperature _____

	ALT I.U.
Position 1	
Position 10	
Position 18	

ALT QUALITY CONTROL

PANEL TWO

Sample No. _____

Storage temperature _____

	ALT I.U.
Position 2	
Position 11	
Position 19	

COMMENTS: _____

V-D. DETECTION OF HEPATITIS B SURFACE ANTIGEN (HBsAG)

PROCEDURES FOR THE USE OF THE
AUSRIA II-125 TEST
(ABBOTT LABORATORIES)

Abbott Laboratories
Diagnostics Division
North Chicago, IL60064

◊ Note Change

ANTIBODY TO HEPATITIS B SURFACE ANTIGEN ^{125}I (HUMAN) AUSRIA® II-125

Radioimmunoassay for the Detection of Hepatitis B Surface Antigen.

This radioactive material may be received, acquired, possessed, and used only by physicians, clinical laboratories, blood banks or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license, or a specific license, of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority

ABBOTT LABORATORIES

Name and Intended Use

Ausria II-125 is Abbott Laboratories' registered trademark for a third generation test for the qualitative radioimmunoassay of Hepatitis B Surface Antigen (HB_sAg) in serum or plasma *

Summary and Biological Principles of the Procedure

The Ausria II-125 system uses a "sandwich principle", a solid phase radioimmunoassay technique, to measure HB_sAg levels in serum or plasma. Plastic beads coated with guinea pig antibody are supplied in

*Plasma may be tested directly only when Procedure B (overnight incubation at room temperature) is employed. If Procedures A or C (45°C procedures) are used, plasma must be *recalcified* prior to testing (see page 5).

the kit. Patient serum or plasma is added and, during incubation, HB_sAg, if present, is fixed to the antibody. When antibody tagged with ^{125}I is added, it binds to any HB_sAg on the bead creating an antibody-antigen-antibody "sandwich".

Within limits, the greater the amount of antigen in the serum specimen, the higher the final count rate.

Explanation of Test

Three Ausria II-125 Procedures with third generation sensitivity are available to identify specimens containing HB_sAg. The Ausria II-125 Procedures A and B (see Performance of Test for Detection of HB_sAg, pages 7-10) are more sensitive than Ausria II-125 Procedure C (see page 13) for detection of HB_sAg. To achieve maximal sensitivity of the Ausria II-125 test system, we recommend the use of Ausria II-125 Procedure A or B. If there is insufficient time to test by Procedure A or B, Ausria II-125 Procedure C is licensed with third generation sensitivity. However, specimens reactive by Procedure C must be further tested to validate the presence of HB_sAg as described under "Interpretation of Results" paragraphs 3 and 4, page 16.

Specimens nonreactive by the Ausria II-125 test are considered negative for HB_sAg and need not be tested further.

Reactive specimens contain either HB_sAg or substances which react nonspecifically. In a small number of cases presumptive reactive specimens result from improper technique.

Screening procedure reactive specimens may be retested by Procedures A or B to determine if they are repeatably reactive. Clinical data indicate that one specimen out of 1000 may be nonrepeatably reactive. If the specimen is repeatably reactive, confirmation testing must be performed. Because of the low incidence of nonrepeatably reactive specimens, confirmation testing may be performed on all specimens initially found reactive by Procedure A or B, thereby eliminating the need for replicate testing.

Confirmation testing for the presence of HB_sAg in Ausria II-125 reactive specimens can be performed in one of two ways. First, specimens can be tested utilizing human Antibody to Hepatitis B Surface Antigen (Anti-HB_s) in neutralization procedures. Second, specimens may be tested with other licensed HB_sAg test systems. If the alternate systems corroborate Ausria II-125 results, the specimens are considered reactive for HB_sAg and no additional testing is required. If there is disagreement between Ausria II-125 and the alternate system, the specimen *must* be confirmed by neutralization testing with human Anti-HB_s using Ausria II-125 Confirmatory Neutralization Test Kit, No. 8310.

Reagents Supplied — Storage Conditions

100 Test Kit: (polystyrene beads packaged in plastic tubes)

Store all reagents at 2° to 8°C when received.

All reagents must be brought to room temperature for use and returned for storage at 2° to 8°C.

For In Vitro Diagnostic Use.

1000 Test Kit: (polystyrene beads packaged in bottles)

Store all reagents at 2° to 8°C when received.

All reagents must be brought to room temperature for use. (Allow 45 to 60 minutes for the beads to equilibrate to room temperature in the tightly stoppered bottles.) The positive control, negative control, and ^{125}I-Anti-HB_s must be returned for storage at 2° to 8°C.

Once a bottle of beads has been brought to room temperature, it must be tightly stoppered after each entry and may be stored at room temperature to eliminate temperature equilibration before each use.

NOTE: Each bottle of beads is identified by a lot number; the lot number of beads in each kit Master Lot is recorded on the kit container. If stored separately, be certain that the bottle of beads is matched to and used with the Master Lot Kit from which it was taken.

For In Vitro Diagnostic Use.

Reagents

1. Negative Human Control (Recalcified human plasma nonreactive for HB_sAg and Anti-HB_s). Preservative: 0.1% sodium azide. Handle as though capable of transmitting hepatitis.
2. Positive Human Control (Human plasma reactive for HB_sAg). 0.01 *M* TRIS Buffer containing 4% Bovine Serum Albumin is used as the diluent to adjust potency to 20 ± 5 ng/ml. Preservative: 0.1% sodium azide. Handle as though capable of transmitting hepatitis. Studies have indicated that the HB_sAg which is present in the Positive Human Control may possibly be a hepatitis agent or be carried in close association with such an agent. Because of this possibility, the Positive Human Control has been heated at 60°C for 10 hours. It is generally accepted that exposure of normal serum albumin to this temperature for this time will inactivate the hepatitis agent. Nevertheless, complete inactivation should not be assumed.
3. Antibody to Hepatitis B Surface Antigen ^{125}I (Human). 0.005 *M* Tristhydroxymethylaminomethane Buffer containing 50% Calf Serum, 2% Normal Human Serum and 0.5% Bovine Serum Albumin is used as the diluent to adjust potency. Activity: 0.74 microcurie or less/ml. Preservative: 0.1% sodium azide. Handle as though capable of transmitting hepatitis.
4. Antibody to Hepatitis B Surface Antigen (Guinea Pig) — Coated beads (polystyrene beads coated with guinea pig Anti-HB_s). Handle as though capable of transmitting hepatitis.

Additional Precautions

I. Storage

The user shall store the radioactive material until used in the original shipping package or in a container providing equivalent radiation protection including the refrigerator properly marked with a radiation hazard sign.

Pursuant to a "Certificate of Registration" received after filing form NRC-483, laboratories may receive products containing iodine-125 in units not exceeding 10 microcuries each, and may not possess at any one time, at any one location of storage or use a total amount of iodine-125 in excess of 200 microcuries. If the laboratory uses several radioiodinated products, all of the unused products on hand may not exceed this amount. The 100 test Ausria II-125 kit contains 14.8 microcuries or less, in units of 7.4 microcuries or less each. Consequently, if the Ausria II-125 kit is the *only* source of radioactivity, a blood bank or laboratory operating under the above certificate may possess no more than thirteen 100 test kits at any time. Since the 1000 test Ausria II-125 kit provides the Antibody to Hepatitis B Surface Antigen ^{125}I (Human) in bottles containing up to 29.6 microcuries each, laboratories wishing to use this kit should apply for a specific license under Section 30.32, USNRC Form 313. Licensees in Agreement States should refer to the appropriate regulations of their own state.

II. Handling

The following precautions should be observed in handling Ausria materials:

1. Handling should preclude any pipetting by mouth.
2. There should be no smoking or eating where radioactive or antigen-containing materials are being handled.
3. Hands should be covered with rubber gloves during, and thoroughly washed after, handling of radioactive materials.
4. Spills should be wiped up quickly and thoroughly with a 5% sodium hypochlorite solution and contaminated materials added to radioactive waste matter.
5. The specimens found to be reactive by the Ausria II-125 test and all materials used to perform the test should be disposed of as if they contained the infectious agent of viral hepatitis. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Rubber gloves worn throughout the entire procedure should also be decontaminated before discarding. Disposable materials may be incinerated. Liquid wastes may be mixed with sodium hypochlorite in volumes such that the final mixture contains 2.5% sodium hypochlorite.* Allow 30 minutes for sterilization to be completed.
6. Certain small quantities of ^{125}I liquid waste may be disposed of through a selected sink drain. Details are available from the

*Cossart, Y., Epidemiology of Serum Hepatitis, Brit. Med. Bull. 28:156, 1972.
Snydman, D.R., Bryan, J.A., and Dixon, R.E., Prevention of Nosocomial Viral Hepatitis, Type B (Hepatitis B), Ann. Int. Med. 83:818, 1975.

Diagnostics Division of Abbott Laboratories, Abbott Park AP-8, North Chicago, Illinois 60064. Reference can be made to Title 10, Code of Federal Regulations, Part 20.

USNRC Form 483 *in vitro* registrants may dispose of solid waste by conventional means.

III. General

1. Do not mix reagents from different master lots.
2. Do not use kit components beyond the expiration date.
3. All reagents should be brought to room temperature before use (handle beads as described under *Reagents Supplied — Storage Conditions*, page 2).
4. When opening and removing aliquots from the primary vials, care should be taken to avoid microbial contamination of reagents.
5. If the Multi-Bead Dispenser is used with the 1000 Test Kit, any unused beads remaining in the Dispenser must be returned to the original container (see instructions accompanying Multi-Bead Dispenser) and tightly stoppered for storage.
6. Reagents should not be exposed to strong light during storage or incubation.

Specimen Collection and Preparation

The Ausria II-125 test may be performed on human serum or plasma.

1. If serum is to be tested, Procedure A, B or C can be used.
2. If plasma is to be tested, Procedure B can be used without prior treatment of specimens. To use Procedure A or C for testing plasma, specimens must be recalcified as described in the next step.
3. Plasma collected into ACD, CPD or 4% citrate solution can be tested by Procedure A or C only after recalcification by the following or equivalent method.
   a. Prepare a 2.77% solution of calcium chloride in water (do not store this solution more than one week).
   b. Add 0.1 ml of the calcium chloride solution to 0.9 ml of plasma and incubate at 37°C for two hours.
   c. Recover recalcified plasma by centrifuging at 500 - 1000 x g for 15 minutes in a clinical centrifuge. An alternate method for recovering the recalcified plasma is freezing the specimens following the 37°C incubation and then allowing to thaw. This retracts the clot so that the recalcified specimen can be recovered without centrifugation.
4. If specimens are to be stored, they should be refrigerated at 2° to 8°C or frozen. Sodium azide to a final concentration of 0.1% w/v may be added to retard biological growth. If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.

Procedure

Materials Provided

No. 7802, Ausria II-125 Kit (100 Tests)

Kit contains:

4 Tubes (25 beads each) polystyrene beads coated with Antibody to Hepatitis B Surface Antigen (Guinea Pig) and one dispensing tip.

2 Vials (10 ml each) Antibody to Hepatitis B Surface Antigen ^{125}I (Human), 0.74 microcurie or less/ml; Preservative: 0.1% sodium azide.

1 Vial (5 ml) Negative Control (Nonreactive for HB_sAg and Anti-HB_s) Preservative: 0.1% sodium azide.

1 Vial (3 ml) Positive Control (Positive for HB_sAg) Preservative: 0.1% sodium azide.

No. 7802, Ausria II-125 (1000 Tests)

Kit contains:

2 Bottles (500 beads each) polystyrene beads coated with Antibody to Hepatitis B Surface Antigen (Guinea Pig).

5 Vials (40 ml each) Antibody to Hepatitis B Surface Antigen ^{125}I (Human), 0.74 microcurie or less/ml; Preservative: 0.1% sodium azide.

1 Vial (35 ml) Negative Control (Nonreactive for HB_sAg and Anti-HB_s) Preservative: 0.1% sodium azide.

1 Vial (16 ml) Positive Control (Positive for HB_sAg) Preservative: 0.1% sodium azide.

Units of the above reagent kit are shipped in accordance with customer order.

An optimum combination of the following accessories is provided for performance of the tests ordered.

Reaction Trays (20 or 60 wells per tray)

Cover Sealers (for 60 well trays; tear along perforation for use with 20 well trays)

Tube Identification Inserts (for groups of 20 or 60 counting tubes)

Counting Tubes with identifying cartons (for transfer of beads from Reaction Trays)

Materials Required but not Provided

1. Precision pipettes or similar equipment to deliver 0.2 ml.
2. No. 6155, Multi-Bead Dispenser, for dispensing twenty beads at one time from a 500 bead bottle into the Reaction Tray wells.
3. Device for delivery of rinse solution such as Cornwall syringe, Filamatic or equivalent.
4. An aspiration device for washing coated beads such as a cannula, aspirator tip, Uniwash™ II or Pentawash* II with a vacuum

TM – Trademark

source and a trap for retaining the aspirate.

5. A well-type gamma scintillation detector capable of efficiently counting ^{125}I.
6. Gently circulating water bath, capable of maintaining temperature at 45° ± 1°C.
7. No. 8310, Antibody to Hepatitis B Surface Antigen (Human) Ausria Confirmatory Neutralization Test Kit.

This radioimmunoassay *must* be performed on all Ausria II-125 repeatably reactive specimens unless they can be confirmed as positive by other licensed HB_sAg test systems. *See Explanation of Test*, page 2.

Accessory Products

Washing Devices:

No. 7693, Uniwash II, washes and aspirates 1 well in a reaction tray. Must be used with appropriate vacuum and dispensing source.

No. 6118, Pentawash II, washes and aspirates 5 wells in a reaction tray at once. Must be used with appropriate vacuum and dispensing source.

Miscellaneous:

No. 6152, Vacuum Pump, for use with No. 7693, Uniwash II; No. 6118, Pentawash II or equivalent.

No. 8969, Dispensing Pump, for dispensing rinse water for use with No. 7693, Uniwash II; No. 6118, Pentawash II or equivalent.

Cornwall Syringe, for dispensing rinse water for use with No. 7693, Uniwash II or equivalent.

Performance of Test for Detection of HB_sAg

Procedure A — (Incubation: 2 hours at 45°C; 1 hour at 45°C)

This procedure can be used to test serum or recalcified plasma.

Seven negative and three positive controls should be assayed with each run of unknowns. Insure that reaction trays containing controls and reaction trays of unknowns are subjected to the same process and incubation times.

CAUTION: Use a clean pipette or disposable tip for each transfer to avoid cross-contamination.

1. If plasma specimens are to be tested, they must be recalcified as described under *Specimen Collection and Preparation*, page 5, prior to assay by Procedure A.
2. Adjust temperature of water bath to 45°C.
3. Dispense one bead for each specimen to be tested.
   a. For 100 Test Kit (polystyrene beads packaged in plastic tubes): Remove cap from clear plastic tube that contains antibody coated beads and attach dispensing tip to the open end. Remove dispensing tip cover and hold bead dispenser directly over top of well in reaction tray. Push down on dispensing tip with index finger to release one bead per well for each sample to be tested.

b. For 1000 Test Kit (polystyrene beads packaged in bottles): NOTE: Beads stored at 2° to 8°C must be allowed to equilibrate to room temperature before use (see *Reagents Supplied — Storage Conditions*, page 2)

Remove cap from the bottle and transfer beads to a Multi-Bead Dispenser (see instructions accompanying Multi-Bead Dispenser). Dispense beads into the wells of the reaction tray so that there is one bead for each specimen to be tested. If fewer than 20 beads are needed, individual beads may be transferred one at a time from the bottle to the reaction wells.

4. Pipette 0.2 ml of specimen † and positive and negative controls to the their respective wells.
5. Apply cover sealer to each tray. Make sure that the bead is completely covered by the specimen. Tap the reaction tray to release any air bubbles trapped in the specimen.
6. Incubate the trays in the 45°C water bath for two hours.
7. At the end of two hours remove the trays from the water bath. Remove the cover sealer and discard. Using a semi-automated aspiration and rinsing system, i.e. Uniwash II or Pentawash II, and an automatic delivery system and vacuum source, follow the directions supplied with the semi-automated system and aspirate the specimen. Rinse each well and bead with 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

A manual system of washing the wells and beads may also be used. Using disposable pipettes or cannulas attached to a vacuum source and a Cornwall syringe delivery system, or equivalent, rinse *each* well and bead. Use extreme care not to overflow the reaction well but assure that the bead is totally immersed throughout the wash procedure. Place the pipette or cannula, attached to the vacuum source, into the bottom of the well next to the bead and slowly add with the Cornwall syringe 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

8. With precision pipettes, add 0.2 ml of ^{125}I-Anti-HB$_s$ (Human) to the bottom of each reaction well.
9. Apply new cover sealer to each tray. Make sure that the bead is completely surrounded by the labeled antibody solution. Tap to release any air bubbles trapped in the solution.
10. Incubate the trays in the 45°C water bath for one hour.
11. At the end of one hour remove the trays from the water bath. Remove cover sealer, aspirate the antibody solution from each well and rinse the well and bead with a total of two 5 ml portions of distilled or deionized water as in Step 7.
12. Transfer beads from reaction wells to properly identified counting tubes: align inverted rack of oriented counting tubes over reaction tray, press tubes tightly over wells, then invert tray and tubes together so that beads fall into corresponding tubes.

†Specimen volume may vary from 0.2 ml to 0.4 ml without affecting the performance of the test.

13. Place the counting tubes in a suitable well type gamma scintillation counter and determine the count rate. Although it is not critical that the counting be done immediately, it is preferable that it be done within 24 hours after the final wash. All control samples and unknowns must be counted together.

See *RESULTS* section, page 10.

Procedure B — (Incubation: Overnight at Room Temperature; 1 hour at 45°C)

This procedure can be used to test serum or plasma.

Seven negative and three positive controls should be assayed with each run of unknowns. Insure that the reaction trays containing controls and reaction trays of unknowns are subjected to the same process and incubation times.

CAUTION: Use a clean pipette or disposable tip for each transfer to avoid cross-contamination.

1. Dispense one bead for each specimen to be tested.
   a. For 100 Test Kit (polystyrene beads packaged in plastic tubes): Remove cap from clear plastic tube that contains antibody coated beads and attach dispensing tip to the open end. Remove dispensing tip cover and hold bead dispenser directly over top of reaction tray well. Push down on dispensing tip with index finger to release one bead per well for each sample to be tested.
   b. For 1000 Test Kit (polystyrene beads packaged in bottles): NOTE: Beads stored at 2° to 8°C must be allowed to equilibrate to room temperature before use (see *Reagents Supplied — Storage Conditions*, page 2).

   Remove cap from the bottle and transfer beads to a Multi-Bead Dispenser (see instructions accompanying Multi-Bead Dispenser). Dispense beads into the wells of the reaction tray so that there is one bead for each specimen to be tested. If fewer than 20 beads are needed, individual beads may be transferred one at a time from the bottle to the reaction wells.
2. Pipette 0.2 ml of specimen† and positive and negative controls to the bottom of their respective wells.
3. Apply cover sealer to each tray. Make sure that the bead is completely covered by the specimen. Tap the reaction tray to release any air bubbles trapped in the specimen.
4. Incubate the trays on a level surface at room temperature for 16 hours (12 to 20 hours).
5. At the end of the incubation period remove the cover sealer and discard. Using a semi-automated aspiration and rinsing system, i.e. Uniwash II or Pentawash II, and an automatic delivery system and vacuum source, follow the directions supplied with

†Specimen volume may vary from 0.2 ml to 0.4 ml without affecting the performance of the test.

the semi-automated system and aspirate the specimen. Rinse each well and bead with 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

A manual system of washing the wells and beads may also be used. Using disposable pipettes or cannulas attached to a vacuum source and a Cornwall syringe delivery system, or equivalent, rinse *each* well and bead. Use extreme care not to overflow the reaction well but assure that the bead is totally immersed throughout the wash procedure. Place the pipette or cannula, attached to the vacuum source, into the bottom of the well next to the bead and slowly add with the Cornwall syringe 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

6. Adjust the temperature of water bath to 45°C.
7. With precision pipettes, add 0.2 ml of ^{125}I-Anti-HB_s (Human) to each reaction well.
8. Apply new cover sealer to each tray. Make sure that the bead is completely covered by the labeled antibody solution. Tap to release any air bubbles trapped in the solution.
9. Incubate the trays in the 45°C water bath for one hour.
10. At the end of one hour remove the trays from the water bath. Remove cover sealer, aspirate the antibody solution from each well and rinse the well and bead with a total of two 5 ml portions of distilled or deionized water as in Step 5.
11. Transfer beads from reaction wells to properly identified counting tubes: align inverted rack of oriented counting tubes over reaction tray, press tubes tightly over wells, then invert tray and tubes together so that beads fall into properly labeled tubes.
12. Place the counting tubes in a suitable well type gamma scintillation counter and determine the count rate. Although it is not critical that the counting be done immediately, it is preferable that it be done within 24 hours after the final wash. All control samples and unknowns must be counted together.

Results Procedures A and B

The presence or absence of HB_sAg is determined by relating net counts per minute†† of the unknown sample to net counts per minute of the negative control mean times the factor 2.1.

Unknown samples whose net count rate is higher than the mean cutoff value established with the negative control are to be considered reactive for HB_sAg.

The mean value for the positive control samples should be at least 5 times the negative control mean. If not, technique may be suspect and the run should be repeated.

††For gamma counters which do *not* automatically subtract machine background, the gross counts may be used if the cutoff value for the negative control is calculated by the method shown in the NOTE, page 11.

Calculation For Determining Cutoff Value

1. Calculation of the negative control mean

   a. Example:

Negative Control Sample No.	Net Count Rate Per Minute
1	190
2	200
3	205
4	188
5	175
6	195
7	200
	Total 1353

$$\frac{\text{Total net cpm}}{7} = \frac{1353}{7} = 193 \text{ net cpm (mean)}$$

   b. Elimination of aberrant values

   Method:

   Discard those individual values in the negative control samples which fall outside of the range 0.5 to 1.5 times the mean.

   Example:

   0.5 × 193 = 97 and 1.5 × 193 = 290

   Range = 97 cpm to 290 cpm

   In the example, no negative control sample is rejected as aberrant.

   c. The negative control mean, therefore need not be revised. Typically all negative control values should fall within the range 0.5 to 1.5 times the negative control mean. If more than one value is consistently found outside this range, technique problems should be investigated.

2. Calculation of the cutoff value (see NOTE)

   a. Multiply the net negative control mean, 193 cpm, by the factor 2.1.

   b. The calculated cutoff value is then 405 cpm.

   c. Unknowns whose net count rate is higher than the cutoff value should be considered reactive with respect to HB_sAg.

NOTE: Many gamma counters have no capacity for automatically subtracting background. In this case, as an alternative to subtracting instrument background manually from each specimen, uncorrected specimen counts per minute can be compared with a cutoff modified as follows:

(Negative control mean − Background) × 2.1 + Background = Cutoff

Example:

Gross negative control mean = 243 cpm

Instrument background = 50 cpm

Cutoff = (243 − 50) × 2.1 + 50 = 455 cpm

Samples with gross count rates greater than 455 cpm are to be considered reactive with respect to HB_sAg.

3. Calculation of positive control: negative control ratio
   a. Divide the positive control mean value by the negative control mean value after correcting for background:

$$\frac{\text{Net Positive Control Mean}}{\text{Net Negative Control Mean}} = \text{P/N Ratio}$$

   b. This ratio should be at least 5 or technique may be suspect and the run should be repeated.

   Example:

   Net positive control mean value = 2953 cpm
   Net negative control mean value = 193 cpm
   P/N Ratio = 2953 ÷ 193 = 15.3
   Technique is acceptable and data should be considered valid.

Interpretation of Results

Repeat testing of a screening procedure reactive specimen will verify whether it is repeatably reactive. In making an evaluation of the data, consideration should be given to the actual test values obtained. 2.1 times the negative control mean is used as the cutoff for single determinations. This value has been selected in order to decrease the total number of nonrepeatably reactive specimens.

If repeat testing shows the specimen to be less than 2.1 times the negative control mean, the original result may be classified as nonrepeatably reactive. If repeats are above the cutoff value, the specimen should be presumed positive for HB_sAg. Such results are contingent on determination of the specificity of the repeatably reactive specimens.

False reactive results may be obtained with any diagnostic test. Two types of false reactive results may occur with Ausira II-125:

1. *Nonrepeatably Reactive Specimens*: Some specimens which are reactive in the Ausria II-125 screening procedure may not be reactive on repeat testing. This phenomenon is highly dependent on technique used in running the test. The most common sources of such nonrepeatably reactive specimens are: a) inadequate rinsing of bead, b) contamination of specimen holders in the gamma counter and c) cross-contamination of nonreactive specimens caused by transfer of residual droplets of high titer, antigen containing sera on the pipetting device.
2. *Nonspecific Reactive Specimens*: The nonspecific falsely reactive specimens which result from cross reactions in the sandwich technique (antibody-antigen-antibody) appear to have been virtually eliminated by using a heterologous antibody system (guinea pig Anti-HB_s coated beads and ^{125}I labeled human Anti-HB_s). All highly sensitive immune systems have a potential for nonspecific reaction, but it is highly unusual to find Ausria II-125 repeatably reactive specimens which cannot be confirmed by the licensed Ausria Confirmatory Neutralization Test Kit, No. 8310.

Specificity analysis must be performed prior to informing a donor that he is an HB_sAg carrier.

Antibody to Hepatitis B Surface Antigen (Human), Kit, No. 8310, provides a method for confirmation of screening procedure reactive specimens. This radioimmunoassay *must* be performed on all repeatably reactive specimens unless they can be confirmed positive by other licensed HB_sAg test systems.

A specimen which is repeatably reactive by the Ausria II-125 test and is confirmed by neutralization with human antibody must be considered positive for HB_sAg.

See *Explanation of Test*, page 2.

Procedure C — (Incubation: 30 minutes at 45°C; 15 minutes at 45°C)

This procedure can be used to test serum or recalcified plasma. It is designed to provide a rapid method for the screening of specimens for HB_sAg when time does not permit testing by Procedure A or B. Procedure C is a third generation test but is less sensitive than Procedures A and B for the detection of HB_sAg positive specimens (see Tables 2, 3 and 4, pages 18-19). The incidence of nonrepeatably reactive specimens may be slightly higher with Procedure C than with Procedures A and B (see Tables 5 and 6, page 20).

Procedure C must be used in conjunction with Procedure A to obtain the negative control mean cutoff value and to ensure a valid run. The same Master Lot of reagents must be used for both Procedure A and Procedure C, and the specimens must be counted on the same gamma counter.

CAUTION: Use a clean pipette or disposable tip for each transfer to avoid cross-contamination.

1. If plasma specimens are to be tested, they should be recalcified as described under *Specimen Collection and Preparation*, page 5, prior to assay by Procedure C.
2. Adjust temperature of water bath to 45°C.
3. Dispense one bead for each specimen to be tested.
   a. For 100 Test Kit (polystyrene beads packaged in plastic tubes): Remove cap from clear plastic tube that contains antibody coated beads and attach dispensing tip to the open end. Remove dispensing tip cover and hold bead dispenser directly over top of well in reaction tray. Push down on dispensing tip with index finger to release one bead per well for each sample to be tested.
   b. For 1000 Test Kit (polystyrene beads packaged in bottles): NOTE: Beads stored at 2° to 8°C must be allowed to equilibrate to room temperature before use (see *Reagents Supplied — Storage Conditions*, page 2).

   Remove cap from the bottle and transfer the beads to a Multi-Bead Dispenser (see instructions accompanying Multi-Bead Dispenser). Dispense beads into the wells of the reaction tray so that there is one bead for each specimen to be tested.

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If fewer than 20 beads are needed, individual beads may be transferred one at a time from the bottle to the reaction wells.

4. Pipette 0.2 ml of specimen† to properly identified wells.
5. Apply cover sealer to each tray. Make sure that the antibody coated bead is completely covered by the specimen. Tap the reaction tray to release any air bubbles trapped in the specimen.
6. Incubate the trays in the 45°C water bath for 30 minutes.
7. At the end of 30 minutes remove the trays from the water bath. Remove the cover sealer and discard. Using a semi-automated aspiration and rinsing system, i.e., Uniwash II or Pentawash II, and an automatic delivery system and vacuum source, follow the directions supplied with the semi-automated system and aspirate the specimen. Rinse each well and bead with 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

   A manual system of washing the wells and beads may also be used. Using disposable pipettes or cannulas attached to a vacuum source and a Cornwall syringe delivery system, or equivalent, rinse *each* well and bead using extreme care not to overflow the reaction well but assure that the bead is totally immersed throughout the wash procedure. Place the pipette or cannula, attached to the vacuum source, into the bottom of the well next to the bead and slowly add with the Cornwall syringe 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.
8. With precision pipettes, add 0.2 ml of ^{125}I-Anti-HB$_s$ (Human) to each reaction well.
9. Apply a new cover sealer to each tray. Make sure that the bead is completely covered by the labeled antibody solution. Tap to release any air bubbles trapped in the solution.
10. Incubate the trays in the 45°C water bath for 15 minutes.
11. At the end of 15 minutes remove the trays from the water bath. Remove cover sealer, aspirate the antibody solution from each well and rinse the well and bead with a total of two 5 ml portions of distilled or deionized water as in Step 7.
12. Transfer beads from reaction wells to properly identified counting tubes: align inverted rack of oriented counting tubes over reaction tray, press tubes tightly over wells, then invert tray and tubes together so that beads fall into corresponding tubes.
13. Place the counting tubes in a suitable well type gamma scintillation counter and determine the count rate. The counting must be performed in the same gamma counter used to count the controls run by Procedure A. Although it is not critical that the counting be done immediately, it is preferable that it be done within 24 hours after the final wash.

†Specimen volume may vary from 0.2 ml to 0.4 ml without affecting the performance of the test.

Results Procedure C

The presence or absence of HB$_s$Ag is determined by relating net counts per minute* of the specimens being tested to net counts per minute of the negative control mean times the factor 1.5, from a test run during the same day using Procedure A. If Procedure A has not been completed by the time the controls are needed for the calculations, the negative control mean obtained from an Ausria II-125 Procedure A test performed during the prior 24 hour period may be used to determine the cutoff value. If these negative control values are not available, it is permissible to use the negative control values determined with Ausria II-125 Procedure A during the prior 72 hour period to calculate the cutoff value.

CAUTION: It is MANDATORY THAT THE SAME MASTER LOT OF AUSRIA II-125 be used for both Procedure A and Procedure C and that the specimens are COUNTED ON THE SAME GAMMA COUNTER to obtain valid results.

When the controls from Procedure A are counted on a different gamma counter, or the counter has been recalibrated or adjusted, the negative controls from Procedure A *must* be recounted with the same gamma counter which is to be used to count the specimens from Procedure C.

To ensure the validity of the results obtained by Procedure C, the same Master Lot of reagents must be used to run the negative and positive controls by Procedure A during the same day in order to determine P/N ratio (see Calculation of positive control: negative control ratio, page 16). The mean value for the positive control specimens from Procedure A should be at least 5 times the negative control mean. If not, the results obtained by Procedure C should not be considered valid.

Calculation for Determining Cutoff Value

1. Calculation of the negative control mean.
   See Section 1, parts a, b and c, page 11.
   NOTE: IT IS IMPORTANT that the negative control mean value from Procedure A should not vary more than ±50% from the average negative control means run during the prior 72 hour period with the same Master Lot of reagents and counted on the same gamma counter. If this occurs, the results from Procedure C should be considered suspect and the discrepancy should be resolved.
2. Calculation of the cutoff value (see NOTE).
   a. Multiply the negative control mean from Procedure A by the factor 1.5 to obtain the cutoff value for Procedure C.
   b. Specimens whose net count rate is equal to or greater than the cutoff value should be considered reactive for HB$_s$Ag by Ausria II-125. The reactive specimens must be further tested to validate the presence of HB$_s$Ag as described under "Interpretation of Results", paragraphs 3 and 4, page 16.

*For gamma counters which do not automatically subtract instrument background, the gross counts may be used if the cutoff value for the negative control is calculated by the method shown in the NOTE, page 16.

NOTE: Many gamma counters have no capacity for automatically subtracting background. In this case, as an alternative to subtracting instrument background manually from each specimen, uncorrected specimen counts per minute can be compared with a cutoff modified as follows:

(Negative control mean from Procedure A − background) × 1.5 + background = Cutoff

Example:
Gross negative control mean = 243 cpm
Instrument background = 50 cpm
Cutoff = (243 − 50) × 1.5 + 50 = 340 cpm

Specimens with gross count rates equal to or greater than 340 cpm are to be considered reactive for HB_sAg until further testing can be done by Procedure A or B.

3. Calculation of positive control: negative control ratio.
   Calculate from the positive and negative control values determined by Procedure A during the same day. See method for Calculation of positive control: negative control ratio, Section 3, page 12.

Interpretation of Results

Specimens whose net count rates from Procedure C are higher than the cutoff value of 1.5 times the negative control mean from Procedure A are to be considered reactive for HB_sAg until further testing can be done by Procedure A or B.

The cutoff value has been selected in order to increase the detectability of HB_sAg reactive specimens by Procedure C with only a moderate increase in the number of nonrepeatably reactive specimens.

Repeat testing of a screening procedure reactive specimen by Procedure A or B will verify whether it is a repeatably reactive specimen. In making an evaluation of the data, consideration should be given to actual test values obtained. If repeat testing by Procedure A or B shows the specimen to be less than 2.1 times the negative control mean, the original result may be classified as nonrepeatably reactive.

If repeats are equal to or greater than the cutoff value of 2.1 times the negative control mean by Procedure A or B, the specimen should be presumed reactive for HB_sAg. Such results are contingent upon determination of the specificity of the repeatably reactive specimens.

False reactive results may be obtained with any diagnostic test. Two types of false reactive results may occur with Ausria II-125:

1. *Nonrepeatably Reactive Specimens:* Some specimens which are reactive in the Ausria II-125 screening procedure may not be reactive on repeat testing. This phenomenon is highly dependent on technique used in running the test. The most common sources of such nonrepeatably reactive specimens are a) inadequate rinsing of bead, b) contamination of specimen holders in the gamma counter and c) cross-contamination of nonreactive specimens caused by transfer of residual droplets of high titer, antigen containing sera on the pipetting device.

2. *Nonspecific Reactive Specimens:* The nonspecific reactive specimens which result from cross reactions in the sandwich technique (antibody-antigen-antibody) appear to have been virtually eliminated by using a heterologous antibody system (guinea pig Anti-HB_s coated beads and ^{125}I labeled human Anti-HB_s). All highly sensitive immune systems have a potential for nonspecific reactions, but it is highly unusual to find Ausria II-125 repeatably reactive specimens which cannot be confirmed by the licensed Ausria Confirmatory Neutralization Test Kit, No. 8310.

Specificity analysis must be performed prior to informing a donor that he is an HB_sAg carrier.

Antibody to Hepatitis B Surface Antigen (Human), Ausria II-125, RIA for the Confirmation of Screening Procedure Reactive Specimens, No. 8310, provides a method for confirmation of repeatably reactive specimens. This radioimmunoassay *must* be performed on all repeatably reactive specimens unless they can be confirmed positive by other licensed HB_sAg test systems.

A repeatably reactive specimen, confirmed by neutralization with human Anti-HB_s or other licensed HB_sAg tests must be considered positive for HB_sAg by Ausria II-125.

See *Explanation of Test*, page 2.

Limitation of the Procedure

Although the association of infectivity and the presence of HB_sAg is strong, it is recognized that presently available methods for HB_sAg detection are not sensitive enough to detect all potentially infectious units of blood, or possible cases of hepatitis.

Expected Results

In random blood donor populations, the number of specimens found repeatably reactive for HB_sAg by Ausria II-125 has typically been less than 1%.

Specific Performance Characteristics

Accuracy: This product meets the requirements for a third generation test when tested against the FDA Reference Panel.

Detectability: The ability of Ausria II-125 Procedures A and B to detect HB_sAg in blood bank donor specimens compared to Ausria II-125 Procedure C, Auscell™ and counterelectrophoresis (CEP) is shown in Table 1. The data include 5,344 plasma units obtained from consecutive blood donors (recalcified prior to testing) and consecutive blood donor serums obtained from a blood bank. All HB_sAg positive specimens detected by CEP were also detected by Ausria II-125 Procedures A, B and C and Auscell.

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Table 1

Ausria II-125 Procedure A and B, Ausria II-125 Procedure C, Auscell, and CEP Detection of HB_sAg in Consecutive Blood Donors

Source of Specimens	Number Tested*	Ausria II 125 Procedures A and B	Ausria II 125 Procedure C	Auscell	CEP
Plasma Units (Volunteer & Commercial)	5344	33 (0.62%)	33 (0.62%)	33 (0.62%)	25 (0.47%)
Blood Bank 1 (Commercial)	20,013	143 (0.71%)	142 (0.71%)	140 (0.70%)	115 (0.57%)
Total	25,357	176 (0.69%)	175 (0.69%)	173 (0.68%)	140 (0.55%)

In these studies:

1. Ausria II-125 Procedures A and B detected 1 more HB_sAg positive than Ausria II-125 Procedure C, 3 more than Auscell and 36 more than CEP.
2. Ausria II-125 Procedures A and B detected about 26% more HB_sAg positive specimens than CEP.
3. Ausria II-125 Procedure C detected about 25% more HB_sAg positive specimens than CEP.
4. In this random donor population, Ausria II-125 Procedure C detected 99.4% of the confirmed HB_sAg positive specimens detected by Ausria II-125 Procedures A and B.

In a collection of 195 Ausria II-125 Procedure A positive specimens which were negative by CEP, 191 (97.9%) specimens were positive by Ausria II-125 Procedure C.

Sensitivity: The relative sensitivity of Ausria II-125 Procedures A, B and C was compared with other HB_sAg test systems. Serial two-fold dilutions of HB_sAg positive human serums and purified antigens (subtypes *ad* and *ay*) were prepared in normal human serum. The maximum serial two-fold dilutions detected by Ausria II-125 Procedures A and B, Ausria II-125 Procedure C, Auscell and rheophoresis (RHEO) are shown in Tables 2, 3 and 4.

Table 2

Maximum Dilution of Serums Detected HB_sAg Positive by Each Test

Serum Identification and Subtype	Ausria II-125 Procedures A and B	Ausria II 125 Procedure C	Auscell	CEP/ RHEO
60009 *(ad)*	1:65,536	1:32,768	1:32,768	1:256
60232 *(ad)*	1:16,384	1:4,096	1:4,096	1:32
GFR *(ad)*	1:32,768	1:8,192	1:16,384	1:128
61193 *(ay)*	1:16,384	1:2,048	1:4,096	1:64
60393 *(ay)*	1:4,096	1:512	1:1,024	1:8
48943 *(ay)*	1:2,048	1:512	1:1,024	1:16

*Reflects number of specimens tested by Ausria II 125 Procedures A and B. Ausria II 125 Procedure C, Auscell and CEP tests were performed on specimens detected and confirmed positive for HB_sAg by Ausria II 125 Procedures A and B.

Table 3

Purified HB_sAg/ad (μg/ml) Detected by Each Test

Concentration (μg/ml)	Ausria II 125 Procedures A and B (cpm/NCE)	Ausria II-125 Procedure C (cpm/NCE)	Auscell (Reciprocal of titer)	CEP/ RHEO (+/0)
5.120	—	64.1	≥ 128	+
2.560	—	56.6	≥ 128	+
1.280	—	42.3	≥ 128	0
0.640	103.8	35.0	≥ 128	0
0.320	79.7	21.9	≥ 128	0
0.160	49.1	12.9	≥ 128	0
0.080	46.1	7.2	128	0
0.040	23.9	4.4	64	0
0.020	11.3	2.9	32	0
0.010	7.2	1.9	16	0
0.005	3.7	1.4	< 16	0
0.0025	2.2	1.1	< 16	0
0.0013	1.7	1.1	< 16	0

Table 4

Purified HB_sAg/ay (μg/ml) Detected by Each Test

Concentration (μg/ml)	Ausria II-125 Procedures A and B (cpm/NCE)	Ausria II-125 Procedure C (cpm/NCE)	Auscell (Reciprocal of titer)	CEP/ RHEO (+/0)
5.120	—	25.9	≥ 128	+
2.560	—	23.6	≥ 128	+
1.280	—	16.9	≥ 128	0
0.640	61.1	9.3	≥ 128	0
0.320	68.6	6.1	≥ 128	0
0.160	39.9	4.4	64	0
0.080	28.7	2.6	32	0
0.040	15.6	2.1	16	0
0.020	11.2	1.4	< 16	0
0.010	5.0	1.1	< 16	0
0.005	3.0	1.1	< 16	0
0.0025	2.7	1.0	< 16	0
0.0013	1.8	0.9	< 16	0

In these studies:

1. Ausria II-125 Procedures A and B were 250 to 1000 times more sensitive than RHEO.
2. Ausria II-125 Procedures A and B were 2 to 16 times more sensitive than Auscell.
3. Ausria II-125 Procedures A and B were 2 to 16 times more sensitive than Ausria II-125 Procedure C.

Specificity: Procedures A and B: The percentage of specimens found reactive with Ausria II-125 Procedures A and B and the percentage

of these reactive specimens which were found to be repeatably reactive were determined by testing 31,319 serums in a clinical investigation performed at seven blood banks. The presence of HB_sAg in the repeatably reactive specimens was confirmed by neutralization with human Anti-HB_s using Ausria II-125 Confirmatory Neutralization Test Kit, No. 8310. The results of these tests are shown in Table 5.

Table 5

Percent of Ausria II-125 Reactive Specimens Detected by Procedures A and B and Confirmed as Positive for HB_sAg

Negative Screen	Positive Screen	Repeatably Positive	Confirmed Positive
31,150	169	127	127
(99.46%)	(0.54%)	(0.41%)	(0.41%)

In these studies:

1. Ausria II-125 Procedures A and B detected 0.13% screening procedure reactive specimens which were not reactive by retesting.
2. In this random donor population, all of the repeatably reactive specimens detected by Procedures A and B were confirmed as positive for HB_sAg by neutralization with human Anti-HB_s using Ausria II-125 Confirmatory Neutralization Test Kit, No. 8310. To date, only six nonconfirmable repeatably reactive specimens have been reported to Abbott Laboratories from 20 million specimens tested by Ausria II-125.

Procedure C: The percentage of specimens found reactive in Ausria II-125 screening Procedure C and the percentage of these reactive specimens found repeatably reactive by Procedure A or B was determined by testing 3925 random donor specimens in fourteen blood centers and 2817 random donor specimens at Abbott Laboratories. The results of these tests are shown in Table 6.

Table 6

Percent of Ausria II-125 Reactive Specimens Detected by Procedure C and Percent Confirmed as Positive for HB_sAg

Negative Screen	Positive Screen	Repeatably Positive	Confirmed Positive
6,620	122	9	9
(98.23%)	(1.81%)	(0.13%)	(0.13%)

In these studies:

1. Procedure C detected 1.68% screening procedure reactive specimens which could not be repeated by Procedure A. This would suggest that the incidence of nonrepeatably screening procedure reactive specimens may be higher with Procedure C than with Procedures A and B.
2. In this random donor population all of the repeatably reactive specimens detected by Procedure C were confirmed by the Ausria II-125 RIA for the Confirmation of Screening Procedure Reactive Specimens.

◊ The process practiced in the use of this product is covered by U.S. Patent No. 4,012,494 owned by Abbott Laboratories. The price of this product includes a royalty for a license to use this product to practice the patented process. Licenses are available at the same royalty rate to practice the process without the purchase of this product from Abbott Laboratories, Diagnostics Division. For further information contact Abbott Laboratories, Diagnostics Division, North Chicago, IL60064.

Abbott Laboratories, North Chicago, IL60064

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V-E. DETECTION OF ANTIBODY TO HEPATITIS B SURFACE ANTIGEN (ANTI-HBs)

PROCEDURES FOR THE USE OF THE
AUSAB TEST
(ABBOTT LABORATORIES)

Abbott Laboratories
Diagnostics Division
North Chicago, IL60064

◊ Note Change

HEPATITIS B SURFACE ANTIGEN ^{125}I (HUMAN) (subtypes *ad* and *ay*) AUSAB

Radioimmunoassay for the Detection of Antibody to Hepatitis B Surface Antigen

This radioactive material may be received, acquired, possessed, and used only by physicians, clinical laboratories, blood banks, or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license, or a specific license, of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

ABBOTT LABORATORIES

Name and Intended Use

Ausab is Abbott Laboratories' registered trademark for a radioimmunoassay kit containing materials for the qualitative detection and semiquantitation of Antibody to Hepatitis B Surface Antigen (Anti-HB_s) in serum or plasma.

Summary and Biological Principles of the Procedure

The Ausab system uses a "sandwich principle," a solid phase radioimmunoassay technique, to measure Anti-HB_s levels in serum or plasma. Plastic beads coated with human Hepatitis B Surface Antigen (HB_sAg) are supplied in the kit. The patient's specimen is

added and, during incubation, antibody, if present, is fixed to the solid phase antigen. When antigen tagged with ^{125}I is added it binds to antibody on the bead, creating a radioactive antigen-antibody-antigen "sandwich."[1,2]

The value of detecting Anti-HB_s is generally focused on (a) epidemiologic factors associated with transmission of HB_sAg and (b) evaluation of the recovery and prognosis of infected patients.

The detection of Anti-HB_s is indicative of a prior immunologic exposure to the antigen. Serial blood sampling of a patient, post infection, would be expected to demonstrate an antigenemia peak followed by the detection of Anti-HB_s in the blood. The appearance of antibody is indicative of the convalescent state and recovery from the acute phase of the disease.

Data presented in Table 4, page 13, and by Lander, Alter and Purcell[6], using radioimmunoassay methodology for the detection of Anti-HB_s, indicate an antibody incidence of greater than 20% in commercial blood donors. Lander, et al.[6], found an antibody detection incidence of 14.4% in voluntary blood donors, 22.6% in commercial donors, 14.8% in blood bank personnel, 11.4% in laboratory personnel and 82.6% in persons given multiple transfusions.

Clinical specimens from hospital patients reveal an incidence of antibody detection ranging from 4.6% to 25.4% (Table 4, page 13).

Persons under 20 years of age have been shown to have an incidence of Anti-HB_s which is significantly less than in older persons[3]. Ginsberg, Conrad, et al.[2], have shown that the incidence of antibody in military recruits was about 5%, about 14% in older soldiers and about 25% in soldiers with a year of military service in Korea.

The relatively high incidence of Anti-HB_s supports the contention that Hepatitis, associated with HB_sAg, is endemic within the populations studied and that it can be transmitted by nonparenteral routes. Lander, et al.[6], have noted that Anti-HB_s was uniquely associated with persons who had a long experience in handling blood products.

There is no solid scientific evidence which supports the contention that the transfusion of antibody-positive, antigen-negative, blood is potentially harmful to the recipient. In addition, Ginsberg, Conrad, et al.[2], were unable to correlate Anti-HB_s titer in lots of standard human serum gamma globulin with protection against icteric serum hepatitis.

Within limits, the greater the amount of antibody in the serum sample the higher the final count rate.

Explanation of Test

The Ausab test is used to identify specimens containing Anti-HB_s, and if desired, to obtain semiquantitative data on the reactive specimens. Specimens with a cpm* rate equal to or greater than 2.1 × NCx with the Ausab test are reactive while those with cpm rates of less than 2.1 × NCx are nonreactive (see Results, page 7). Nonreactive specimens are considered to be negative for Anti-HB_s by this system and need not be further tested.

*cpm = counts per minute

Reactive specimens contain either Anti-HB_s or occasionally substances which react nonspecifically in the Ausab system. Approximately 1.1 – 1.4 (0.9804) of the specimens tested with the screening procedure were nonrepeatably reactive. These presumptive reactive specimens usually result from improper technique.

Specimens reactive in the screening test should be further tested in one of two ways. First, replicate testing may be performed to verify the reactivity of the specimen. If the specimen is not repeatably reactive, additional testing is not required. Second, if it is desirable to know quantitative approximations of Anti-HB_s present, replicate testing should include a 1:100 dilution. Repeatably reactive specimens may be considered positive by the Ausab test. These specimens can be confirmed by the Ausab Confirmatory Neutralization Test, No. 7594.

Reagents Supplied — Storage Conditions

This product meets requirements when tested against FDA Reference Panel.

Store at 2° to 8°C.
For In Vitro Diagnostic Use

Reagents

1. Negative Human Control (recalcified normal human plasma, nonreactive for HB_sAg and Anti-HB_s). Preservative: 0.1% sodium azide. Handle as though capable of transmitting hepatitis.
2. Positive Human Control (recalcified normal human plasma, reactive for Anti-HB_s). Recalcified normal human plasma is used as the diluent to adjust potency to 512 ± 200 RIA units/ml. Preservative: 0.1% Sodium Azide. Handle as though capable of transmitting hepatitis.
3. Hepatitis B Surface Antigen ^{125}I (Human) (subtypes *ad* and *ayr*) 0.01 *M* Trishydroxymethylaminomethane containing 20% recalcified normal human plasma and 1% bovine serum albumin is used to adjust potency. Activity: 0.74 microcurie or less/ml. Preservative: 0.1% Sodium Azide. Handle as though capable of transmitting hepatitis.
4. Polystyrene Beads coated with HB_sAg (Human) (subtypes *ad* and *ayr*). Handle as though capable of transmitting hepatitis.

Additional Precautions

1. Storage

The user shall store the radioactive material until used in the original shipping container or in a container providing equivalent radiation protection including the refrigerator properly marked with a radiation hazard sign.

Laboratories receiving Ausab under a "Certificate of Registration" under Section 31.11 of the NRC Regulations may have on their premises at one time a maximum of 200 microcuries of radioactive iodine. This means a total of all such products. If the laboratory uses several radioiodinated products, all of the unused products on hand may not exceed this amount. The 100 test Ausab kit contains 14.8 micro-

curies or less. Consequently, if the Ausab kit is the *only* source of radioactivity, a blood bank or a laboratory operating under the above certificate may possess not more than thirteen 100 test kits at any time.

II. Handling

The following precautions should be observed in handling Ausab materials:

1. Handling should preclude any pipetting by mouth.
2. There should be no smoking or eating while radioactive or antigen containing materials are being handled.
3. Hands should be covered with rubber gloves during, and thoroughly washed after handling of radioactive or potentially infectious materials.
4. Spills should be wiped up quickly and thoroughly and contaminated materials added to radioactive waste matter.
5. Certain small quantities of ^{125}I liquid waste may be disposed of through a selected sink drain. Details are available from the Diagnostics Division of Abbott Laboratories, Abbott Park AP-8, North Chicago, Illinois 60064.

   Reference can be made to Title 10, Code of Federal Regulations, Part 20.

   USNRC Form 483 *in vitro* registrants may dispose of solid waste by conventional means.
6. The specimens found to be reactive by the Ausab test and all materials used to perform the test should be handled and disposed of as if they contained the infectious agent of viral hepatitis. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Rubber gloves worn throughout the entire procedure should also be decontaminated before discarding. Disposable materials may be incinerated. Liquid waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 2.5% sodium hypochlorite[5]. Allow 30 minutes for sterilization to be completed.
7. To avoid microbial contamination of reagents, aseptic techniques should be used in removal of aliquots from the primary vials. If the reagents are to be used within 48 hours, seals and stoppers of the primary vials may be removed and the contents may be utilized providing aseptic technique is employed.

III. General

Do not mix materials from different master lots.

Do not use kit components beyond the expiration date.

All materials should be brought to room temperature before use.

Materials should not be exposed to strong light during storage or incubation. This product meets requirements when tested against the FDA Reference Panel.

Specimen Collection and Preparation

Serum and plasma specimens can be tested by the Ausab procedure. If specimens are to be stored they should be refrigerated at 2° to 8°C or frozen. If specimens are to be shipped they should be packed in compliance with Federal Regulations covering the transportation of etiologic agents.

Procedure

Materials Provided

No. 7554, Ausab kit 100 tests*

Kit contains:

- 4 Tubes (25 beads each) Polystyrene Beads coated with Hepatitis B Surface Antigen (Human) (subtypes *ad* and *ayr*)
- 2 Vials (10 ml each) Hepatitis B Surface Antigen ^{125}I (Human) (subtypes *ad* and *ayr*), activity: 0.74 μCi or less/ml; Preservative: 0.1% Sodium Azide.
- 1 Vial (10 ml) Negative Control (nonreactive for Anti-HB$_s$ and HB$_s$Ag); Preservative: 0.1% Sodium Azide.
- 1 Vial (3 ml) Positive Control (reactive for Anti-HB$_s$); Preservative: 0.1% Sodium Azide.

No. 8811, 5 Reaction Trays (20 wells each), 10 Sealers and 100 Tube Identification Inserts accompany this kit.

No. 8812, Counting Tubes (5 cartons, 20 tubes each, properly positioned for transfer of beads from Reaction Trays) accompany this kit.

Materials Required But Not Provided

Oxford precision pipette and disposable tips to deliver 0.2 ml, or equivalent.

No. 7621, Micropipette to deliver 2 μl, or equivalent.

No. 7659, Micropipette Tips to deliver 2 μl, or equivalent.

Device for delivery of rinse solution such as Cornwall syringe, FiLomatic or equivalent.

An aspiration device for washing coated beads such as a cannula, aspirator tip, Uniwash™ II or Pentawash™ II with a vacuum source and a trap for retaining the aspirate.

A well type gamma scintillation detector capable of efficiently counting ^{125}I.

Disposable rubber gloves.

Accessory Products

Washing Devices:

No. 7693, Uniwash II, washes and aspirates 1 well in a reaction tray.

*The actual number of random donor specimens which can be tested with this kit is a function of the number of specimens screened reactive by the Ausab procedure. Tests on reactive specimens must be repeated to determine the number which contain Anti-HB$_s$ by the Ausab procedure. In the populations studied, the number of Ausab positive specimens determined during initial screening tests ranged from 5.0% to 27.1%.

™ = Trademark

Must be used with appropriate vacuum and dispensing source.

No. 6118, Pentawash II, washes and aspirates 5 wells in a reaction tray at once. Must be used with appropriate vacuum and dispensing source.

Miscellaneous:

No. 6152, Vacuum Pump, for use with No. 7693, Uniwash II, No. 6118, Pentawash II or equivalent

No. 6153, Dispensing Pump, for dispensing rinse water for use with No. 7693, Uniwash II, No. 6118, Pentawash II or equivalent

Performance of Test for Detection of Anti-HB$_s$

Seven negative and three positive controls must be assayed with each run of unknowns. Insure that reaction trays containing controls and unknowns are subjected to the same process and incubation times.

CAUTION: Use a clean pipette or disposable tip for each transfer to avoid cross-contamination.

1. Remove cap from clear plastic tube that contains antigen coated beads. Hold bead dispenser directly over top of reaction tray incubation well and push down with index finger to release one bead into a well for each specimen or control sample to be tested.
2. Using precision pipettes, add 0.2 ml of serum or plasma and positive and negative controls to the bottom of their respective reaction wells. Tap the reaction tray to assure complete distribution of the liquid over the beads.
3. Apply a cover sealer to each tray and incubate on a level surface at room temperature for 18 hours (16 to 20 hours).
4. At the end of the incubation period remove the cover sealer and discard. Using a semiautomated aspiration and rinsing system, i.e., Uniwash II or Pentawash II and an automatic delivery system and vacuum source, follow the directions supplied with the semiautomated system and aspirate the serum; rinse each well and bead with 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

   A manual system of washing the wells and beads may also be used. Using disposable pipettes or cannulas and a Cornwall syringe delivery system, or equivalent, and a vacuum source, rinse *each* well and bead using extreme care not to overflow the reaction well but assure that the bead is totally immersed throughout the wash procedure. Place the pipette or cannula, attached to the vacuum source, into the bottom of the well next to the bead and simultaneously slowly add with the Cornwall syringe 5 ml of distilled or deionized water. Repeat this wash procedure one time for a total rinse volume of 10 ml.

5. With precision pipettes, add 0.2 ml of ^{125}I-HB$_s$Ag (Human) to the bottom of each reaction well. Check to insure that the antigen coated bead is completely surrounded by the labeled antigen solution. Tap to release any air bubbles that may be trapped in the solution.
6. Apply a new cover sealer to each tray and incubate the trays on a level surface at room temperature for 4 hours (225 to 255 minutes).
7. At the end of the incubation period remove the cover sealer and discard. Aspirate the antigen solution from each well and rinse the well and antigen coated bead it contains with a total of 10 ml of distilled or deionized water as in Step 4.
8. Transfer beads from reaction wells to properly identified counting tubes: align inverted rack of oriented counting tubes over reaction tray, press tubes tightly over wells, then invert tray and tubes together so that beads fall into properly labeled tubes.
9. Place the counting tubes in a suitable well type gamma scintillation counter and determine the count rate. The position of the bead at the bottom of the counting tube is not important. Although it is not critical that the counting be done immediately, preferably, it should be done within 24 hours after the final wash. All control samples and unknowns must be counted together.

Results of Detection Procedure

Results are based on net cpm. For gamma counters which do *not* automatically subtract instrument background, the gross counts may be used if the cutoff value for the negative control is calculated by the method shown in the NOTE, page 8.

The mean value for the positive control samples should be at least 150 times the negative control mean. If not, technique may be suspect and the run should be repeated.

Reactive and nonreactive specimens are determined by relating net counts per minute of the unknown to net counts per minute of the negative control mean times the factor 2.1 (cutoff value, see example, page 9).

Unknown specimens whose net count rate is lower than the cutoff value established with the negative control are nonreactive and Anti-HB$_s$ negative by the Ausab procedure.

Unknown specimens whose net count rates are higher than the mean cutoff value established with the negative control are reactive with respect to Anti-HB$_s$.

Reactive specimens detected in the initial test must be retested to validate the presence of Anti-HB$_s$ in the specimen by the Ausab procedure.

Calculation For Determining Cutoff Value

1. Calculation of the negative control mean

   a. Example

Negative Control Sample No	Net Count Rate Per Minute
1	106
2	325
3	98
4	151
5	102
6	147
7	138
	Total 1067

$$\frac{\text{Total cpm}}{7} = \frac{1067}{7} = 152 \text{ cpm (mean)}$$

   b. Elimination of aberrant values

   Method: Discard those individual values in the negative control samples which fall outside of the range 0.5 to 1.5 times the mean.

   Example: 0.5 × 152 to 1.5 × 152 = 76 cpm to 228 cpm (range)*

   *In the example 1-a, control sample number 2 (325 cpm) is rejected as aberrant.

   c. The revised negative control mean is $\frac{1067 - 325}{7 - 1} = 124$ cpm.

   Typically, all negative control values should fall within the range 0.5 to 1.5 times the control mean. If more than one value is consistently found outside this range, technique problems should be investigated.

2. Calculation of the Cutoff Value

   a. Multiply the negative control mean, 124 cpm, by the factor 2.1.

   b. The calculated cutoff value is then 260 cpm.

   NOTE: Many gamma counters have no capacity for automatically subtracting background. In this case, as an alternative to subtracting instrument background manually from each sample, uncorrected sample counts per minute can be compared with a cutoff modified as follows:

   Cutoff = (Negative control mean − Instrument background) × 2.1 + Instrument background

   Example:

   Gross negative control mean = 174 cpm
   Instrument background = 50 cpm
   Cutoff = (174 − 50) × 2.1 + 50 = 310 cpm

   Samples with gross count rates greater than 310 cpm are to be considered reactive with respect to Anti-HB$_s$.

3. Calculation of Positive Control/Negative Control Ratio

   a. Divide the positive control mean value (cpm) by the negative control mean value, after correcting for background (cpm).

   $$\frac{\text{Net Positive Control Mean (cpm)}}{\text{Net Negative Control Mean (cpm)}} = \text{P/N Ratio}$$

   b. This ratio should be at least 15.0 or technique may be suspect and the run should be repeated.

   Example:

   Net positive control mean = 2500 cpm

   Net negative control mean = 124 cpm

   Ratio = 2500/124 = 20.2

   Technique is acceptable and data should be considered valid

Interpretation of Results *

Further testing of the specimen in question will verify whether it is repeatably reactive. In making an evaluation of data, consideration should be given to the actual test values obtained. For single determinations, 2.1 times the negative control mean is used as the cutoff value. This value has been selected in order to decrease the total number of nonrepeatably reactive specimens. If repeat testing shows the specimen to be less than 2.1 times the negative control mean, the original result is classified as a nonrepeatably reactive specimen. If repeats are above the cutoff value, the specimen is reactive for Anti-HB$_s$ by the Ausab test.

Specimens which repeatably give counts above the cutoff can be confirmed by the Ausab Confirmatory Neutralization Test, No. 7594. This procedure identifies the occasional nonspecific specimen.

Performance of Test for Semiquantitation of Anti-HB$_s$ (Optional)

The Ausab procedure was used to test several concentrations of the positive control with several master lots of reagents to construct Table 9, page 16 for estimating the concentration of Anti-HB$_s$ in unknown specimens. It was determined in clinical evaluations that by using this table the multiple differences (greater or lesser) in the estimated RIA units was within two-fold of the actual determined RIA units. To use this table, proceed according to the directions outlined below

* For the purposes of the TTV Study, results ≤2.0 are negative; results >10.0 are questionable and should be retested as test kits become available.

Retest the specimen undiluted (0.2 ml serum or plasma) and diluted 1:100* in negative control (for instance 2 µl of sample added to 0.2 ml negative control) by the Ausab procedure, see pages 5-8.

*CAUTION: Extreme care must be employed in the preparation and utilization of the 1:100 dilution. It is recommended that the pipette tip carrying the unknown specimen be placed directly into the negative control in the reaction well, before delivering the contents. The tip should then be flushed out at least 3 times with the negative control unknown mixture to insure a quantitative transfer. Mix the contents of the reaction well by gently tapping.

Results of Semiquantitation Procedure

The estimated RIA Unit value is determined by calculating the ratio of the reactivity of the unknown specimen to the positive control and selecting the estimated RIA Unit value for this ratio from Table 9.

Calculate the ratio, X, for the undiluted specimen *and* for the 1:100 dilution.

$$\text{Ratio (X)} = \frac{\text{Sample (cpm)} - \overline{\text{NC}}\text{x (cpm)}}{\overline{\text{PC}}\text{x (cpm)} - \overline{\text{NC}}\text{x (cpm)}}$$

After computing X values for all undiluted and 1:100 specimens, refer to Table 9 for estimating the RIA Unit concentration. Table 9 contains two columns. The left column is a listing of ratios comparing specimens to the positive control (X values). The right column contains the estimated RIA Units for these corresponding ratios. It is anticipated that in almost every instance either an undiluted or 1:100 specimen will yield a ratio which should be located in the left column. If the diluted specimen ratio was used, the RIA Unit value must be multiplied by the dilution factor to correct the final concentration.

As an aid in helping to make the decision of which value to use (undiluted or 1:100) in reading from the table, a decision table (Table 8, page 15) has been constructed.

Example: Calculation for estimated RIA Units:

Net Sample (cpm) 1:100	455
Net Positive Control (cpm)	2500
Net Negative Control (cpm)	124

$$\text{Ratio} = \frac{455 - 124}{2500 - 124} = 0.14$$

From Table 9, page 16, a value of 0.14 is equivalent to 54 RIA Units. However, a 1:100 dilution was used so the final RIA Unit concentration would be:

$$100 \times 54 = 5400 \text{ RIA Units}$$

NOTE: When reading RIA Units from Table 9, simply use the ratio closer to the calculated ratio of the unknown.

Limitations of the Procedure

False positive results may be obtained with any diagnostic test. Two types of false reactive results may occur with Ausab:

1. *Nonrepeatably Reactive Specimens:* Some of the Ausab reactive specimens may test nonreactive on repeat. This phenomenon is highly dependent on technique used in running the test. The most common sources of such nonrepeatably reactive tests are: a) inadequate rinsing of the bead and, b) cross-contamination of nonreactive specimens caused by transfer of residual droplets of high titer, antibody-containing sera on the pipetting device.
2. *Nonspecific Reactive Specimens:* All sensitive immune systems have a potential for false positives due to nonspecificity of cross reacting substances or components in human serum or plasma. Although nonspecific reactive specimens have not occurred in studies to date, the potential for nonspecific reaction is still present.

   Occasional reactive specimens which cannot be neutralized have occurred in random donor populations. The scientific explanation for these reactions is not clear at this time (see Specificity, page 14).

Specific Performance Characteristics

Sensitivity:

The relative sensitivities of the Ausab and passive hemagglutination (PHA) systems for the detection of Anti-HB_s in immune serums were determined by preparing serial two-fold dilutions of the hepatitis B immune serums in Ausab "Negative Control" (recalcified, normal human plasma nonreactive for HB_sAg and Anti HB_s). These results are shown in Table 1. A comparison of the sensitivity of Ausab and Rhesphoresis (Rheo) was made by testing serial two fold dilutions in Ausab "Negative Control" of human immune serums containing Anti HB_s (subtypes *ad* and *ay*). These results are shown in Table 2.

Table 1

Maximum Dilution for Serums Detecting Anti-HB_s by Ausab and PHA

Sample Identification	Ausab	PHA	
		Anti-*ay*	Anti-*ad*
F (Human)	1:128,000	1:16,000	1:32,000
H (Human)	1:256,000	1:8,000	1:32,000
R (Human)	1:128,000	1:16,000	1:64,000
Chimp	1:128,000	1:32,000	1:16,000
Guinea Pig	1:32,000	1:16,000	1:64,000
Guinea Pig (*ay*) 3961-236	>1:512,000	1:512,000	1:32,000
Guinea Pig (*ad*) 3961-238	1:512,000	1:64,000	1:128,000

Table 2

Maximum Dilution of Human Anti-HB$_s$ Detected by Ausab and Rheophoresis

Dilution	F		H		R	
	Ausab (S/N)[a]	Rheo (+/0)	Ausab (S/N)	Rheo (+/0)	Ausab (S/N)	Rheo (+/0)
Undiluted	155.6	+	111.7	+	125.9	+
1:2	168.7	+	111.4	+	131.2	+
1:4	N.T.[b]	+	N.T.	+	N.T.	+
1:8	N.T.	+	N.T.	+	N.T.	+
1:16	N.T.	+	N.T.	+	N.T.	+
1:32	N.T.	+	N.T.	+	N.T.	+
1:64	N.T.	+	N.T.	+	N.T.	+
1:100	161.5	0	119.7	0	130.0	0
1:1000	97.6	0	89.9	0	91.2	0
1:2000	68.2	0	51.2	0	71.1	0
1:4000	41.3	0	21.2	0	38.7	0
1:8000	23.4	0	15.9	0	20.1	0
1:16000	15.2	0	10.6	0	9.1	0
1:32000	6.9	0	7.2	0	4.2	0
1:64000	3.4	0	4.2	0	2.2	0
1:128000	2.3	0	2.6	0	1.4	0
1:256000	1.7	0	1.5	0	1.0	0
1:512000	1.1	0	1.3	0	0.9	0
1:1,000,000	0.8	0	1.1	0	1.1	0

a-Sample cpm/NC$\bar{x}$
b-Not tested

In these studies:

1. Ausab was 2 to 8-fold more sensitive than PHA.
2. No significant prozoning occurred with these high titer human specimens.
3. Ausab was 1000 to 2000 fold more sensitive than Rheo

Detectability:

The relative detectability of Ausab, PHA and Rheo was determined by testing a panel of 143 confirmed Anti-HB$_s$ positive specimens. These results are shown in Table 3.

Detectability levels of Anti-HB$_s$ were determined by testing 3,554 specimens from several blood donor populations (commercial and volunteer) and routine clinical specimens from prisoners. In addition, 1,045 specimens from hospital patients were tested for the presence of Anti-HB$_s$. These data are shown in Table 4.

The distribution by ratio of specimen cpm to NC$\bar{x}$ (S/N) was determined with 878 Anti-HB$_s$ positive specimens detected in a population of 5,205 consecutive blood donors. These data are shown in Table 5

Table 3

Detection of 143 Confirmed Anti-HB$_s$ Positive by Ausab, PHA and Rheophoresis

Ausab	PHA	Rheophoresis
143/143	139/143	3/143

Table 4

Detection and Confirmation of Anti-HB$_s$ in Serum

Sample Source	Number Tested	Screening Reactive (%)	Repeatably Reactive (%)	Confirmable Positive (%)
Blood Center 1 (Commercial)	2330	482(20.7)	441(18.9)	441(18.9)
Blood Center 2 (Commercial)	1113	269(24.2)	239(21.5)	239(21.5)
Clinical Specimens 1 (Routine/Prisoner)	111	9(8.1)	9(8.1)	9(8.1)
Clinical Specimens 2 (Hospital Patients)	495	23(4.6)	23(4.6)	23(4.6)
Clinical Specimens 3 (Hospital Patients)	382	103(27.0)	97(25.4)	97(25.4)
Clinical Specimens 4 (Hospital Patients)	168	46(27.4)	39(23.2)	39(23.2)
Totals	4599	932(20.3)	848(18.4)	848(18.4)

Table 5

Distribution by S/N Ratio of Anti-HB$_s$ Positive Specimens Detected in a Population of 5205 Consecutive Blood Donors by Ausab

S/N Range	No Screening Reactive (%)	No Repeatably Reactive (%)	No Confirmable Positive (%)
2.1 - 5.0	55(6.3)	11(1.5)	11(1.5)
5.1 - 10.0	35(4.0)	27(3.3)	27(3.3)
10.1 - 20.0	78(8.9)	74(9.0)	74(9.0)
20.1 - 30.0	102(11.6)	102(12.4)	102(12.4)
30.1 - 50.0	316(36.0)	316(38.4)	316(38.4)
50.1 - 70.0	176(20.0)	176(21.4)	176(21.4)
70.1 - 80.0	52(5.9)	52(6.3)	52(6.3)
80.1 - 90.0	26(3.0)	26(3.2)	26(3.2)
90.1 - 100.0	18(2.1)	18(2.2)	18(2.2)
100	20(2.3)	20(2.4)	20(2.4)
Total	878(100)	822(100)	822(100)

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In these studies:

1. Ausab detected 4 more Anti HB_s positive specimens than PHA and 140 more than Rheo in a panel of 143 Anti HB_s positive specimens.
2. Ausab detected Anti HB_s in 4.6% to 25.1% of the populations tested.
3. In testing 9,804 specimens only 1.4% (140/9804) nonrepeatably reactive specimens were detected.
4. In Table 5, out of 56 nonrepeatably Ausab reactive specimens, 52 (91.9%) exhibited S/N ratios of < 10.0.

Specificity:

The specificity of the Ausab system was determined by testing 10,000 specimens from several donor populations and 1,156 clinical specimens from outpatients and hospitalized patients. A summary of confirmatory neutralization studies performed on the Ausab repeatably reactive specimens among these 11,164 is shown in Table 6.

Table 6

Confirmatory Neutralization Studies of Repeatably Reactive Specimens from Random Donor Populations and Clinical Specimens

Population	Number Tested	Number Repeatably Reactive (%)	Number Confirmed (%)	Number Not Confirmed (%)
Blood Center 1	5250	1025(19.5)	1018(19.4)	7(0.1)
Blood Center 2	1315	311(23.7)	308(23.4)	3(0.2)
Blood Center 3	2330	441(18.9)	441(18.9)	0(0.0)
Blood Center 4	1113	239(21.5)	239(21.5)	0(0.0)
Clinical Specimens	1156	168(14.5)	168(14.5)	0(0.0)
Totals	11164	2184(19.6)	2174(19.5)	10(0.09)

In these studies:

1. In these populations, an average of 0.09% of the specimens tested could not be confirmed.
2. It has not been possible to determine the exact nature and significance of these occasional nonconfirmably reactive specimens with the available scientific information, but these specimens are readily identified with the Ausab Confirmatory Neutralization Test Kit, No. 7594.

Semiquantitative Procedure:

Anti-HB_s positive specimens obtained from blood bank populations and field studies (377) were assayed and *estimated* RIA Unit values were obtained by the procedures outlined on pages 6 and 9. The *actual* RIA Unit values were obtained for each repeatably reactive

specimen by determining the highest two fold serial dilution detectable by the Ausab procedure. The data are reported in Table 7.

Table 7

Comparison of Estimated RIA Units with Determined RIA Units

Sample Source	Number of Anti HB_s Positives Tested	Comparison of Estimated and Actual RIA Units: Field Investigators	Abbott
Blood Center 1 (Commercial)	43	a	±1.8[b]
Blood Center 2 (Commercial)	150	a	±1.9
Blood Center 3 (Commercial)	53	±2.1	±1.9
Clinical Samples 1 (Routine)	9	±1.9	±1.8
Clinical Samples 2 (Hospital Patients)	25	±1.9	±1.9
Clinical Samples 3 (Hospital Patients)	97	±1.8	±1.9

a—Tested only at Abbott Laboratories
b—Average multiple difference (greater or less) of estimated RIA Units from actual RIA Units

In these studies:

1. The estimated RIA Unit values determined by Abbott and the field investigators compared favorably with the actual RIA Unit values obtained.
2. The average multiple difference between the estimated and actual RIA Unit values in the populations studied was within a two-fold range.

Table 8

Decision Table

Undiluted Specimen		1:100 Specimen		
cpm NC's ≥2.1	cpm PC's ≥1.0	cpm NC's ≥2.1	cpm PC's ≥1.0	Conclusion
Yes	Yes/No	No	No	Use undiluted specimen
Yes	Yes	Yes	No	Use 1:100 specimen
Yes	No	Yes	No	Read each value from table and average
Yes	Yes	Yes	Yes	Specimen requires further dilution
No	No	Yes/No	Yes/No	Specimen considered negative

Results obtained these 4 Counts could be estimated without further action

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Table 9

Estimation of RIA Unit Concentration

Sample (cpm) − NCE (cpm) / PCE (cpm) − NCE (cpm)	Estimated RIA Units†
1.0	512
0.95	477
0.9	442
0.85	412
0.8	382
0.75	352
0.7	322
0.65	292
0.6	262
0.55	235
0.5	208
0.45	183
0.4	158
0.35	135
0.3	112
0.25	92
0.2	72
0.15	54
0.1	36
0.05	16
0.02	8

†Multiply the estimated RIA Units by the dilution factor to obtain the final RIA Unit concentration.

NOTE: To obtain approximate dilution of specimen in which Anti-HB_s can be detected by Ausab, use the following formula:

RIA Units/16 = Estimated dilution titer of the specimen

This product meets requirements when tested against FDA Reference Panel for Anti-HB_s.

Bibliography

1. Coller, J., Millman, I., Hallberr, T., and Blumberg, B., Australia Antigen and Human Anti AU (1) Detection by Radioimmunoassay. Fed. Proc. 30:354, 1971.
2. Ginsberg, A., Conrad, M., Bancroft, W., Ling, C. and Overby, L., Antibody to Australia Antigen: Detection with a Simple Radioimmune Assay, Incidence in Military Populations and Role in the Prevention of Hepatitis B with Gamma Globulin. J. Lab. Clin. Med. 82(2):317, 1973.
3. Hollinger, F., Vernadum, V. and Dreesman, G., Assay of Australia Antigen and Antibody Employing Double Antibody and Solid Phase Radioimmunoassay Technique and Comparison with the Passive Hemagglutination Methods. J. Immunol. 107:1099, 1971.
4. Hollinger, F., Dreesman, G., Morrison, M. and Melnick, J., Improved Double Antibody Radioimmunoassay (RIA-DA) Methodology for Detecting Hepatitis B Antigen (HBAg) and Antibody (HBAb). Abst., Am Soc. Microbiol. 72:213, 1972.
5. Lander, J., Giles, J., Purcell, R. and Krugman, S., Viral Hepatitis Type B (MS 2 Strain). Detection of Antibody after Primary Injection. New Eng. J. Med. 285:303, 1971.

6. Lander, J., Alter, H. and Purcell, R., Frequency of Antibody to Hepatitis Associated Antigen as Measured by a New Radioimmunoassay Technique. J. Immunol. 106:1164, 1971.
7. Lewis, T., Alter, H., Chalmers, T., Holland, P., Purcell, R., Alling, D., Young, D., Frenkel, L., Lee, S. and Lamson, M., A Comparison of the Frequency of Hepatitis B Antigen and Antibody in Hospital and Non-Hospital Personnel. New Eng. J. Med. 289:647, 1973.
8. Ling, C., and Overby, L., Prevalence of Hepatitis B Virus Antigen as Revealed by Direct Radioimmune Assay with ^{125}I Antibody. J. Immunol. 109:634, 1972.
9. Walsh, J., Yalow, R., and Berson, S., Detection of Australia Antigen and Antibody by Means of Radioimmunoassay Techniques. J. Infect. Dis. 121:550, 1970.
10. Cossart, Y., Epidemiology of Serum Hepatitis. Brit. Med. Bull. 28:156, 1972.
11. Lander, J. J., Holland, P. V., Alter, J. H., et al., Antibody to hepatitis associated antigen. Frequency and pattern of response as detected by radioimmunoprecipitation. J.A.M.A., 220:1079 (1972).

Additional References

Aach, R., Hocker, Jr., E. and Parker, C., Radioimmunoassay for Detection of Hepatitis Associated Antigen (HBAg) and Antibodies to HBAg. *Hepatitis and Blood Transfusion*, Vyas, G., Editor, Grune and Stratton, pp. 155-165, 1972.

Coller, Jr., Millman, I., Hallberr, T. and Blumberg, B., Radioimmunoprecipitation Assay for Australia Antigen, Antibody and Antigen-Antibody Complexes. Proc. Soc. Exp. Biol. Med. 138:249, 1971.

Das, P., Hopkins, R., Cash, J. and Cummings, R., Rapid Identification of Hepatitis Associated Antigen and Antibody by Counter-Electrosomopheresis. Brit. J. Haematol. 21:673, 1971.

Peterson, M. and Barker, L., Detection of Hepatitis-Associated Antigen and Antibody. Bacteriol. Proc. p. 168, 1971.

Puleuky, H. and Olson, C., The Incidence and Significance of Antibody to Australia Antigen in Blood Donors. Am. J. Clin. Pathol. 56:129, 1971.

Wallace, J., Milne, G. and Barr, A., Total Screening of Blood Donations for Australia (Hepatitis Associated) Antigen and its Antibody. Brit. Med. J. 1:663, 1972.

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V-F. DETECTION OF ANTIBODY TO HEPATITIS B CORE ANTIGEN (ANTI-HBc)

PROCEDURES FOR THE USE OF THE

CORAB TEST

(ABBOTT LABORATORIES)

The Abbott procedure will be performed as specified in the CORAB package insert. The test results will be reported as percent inhibition, calculated as follows:

$$\frac{\text{Net sample cpm}}{\text{Net Neg. control mean}} \times 100 = \% \text{ S/N}$$

ANTIBODY TO HEPATITIS B CORE ANTIGEN ^{125}I (HUMAN)/HEPATITIS B CORE ANTIGEN (HUMAN) CORAB™

Radioimmunoassay for the Detection of Antibody to Hepatitis B Core Antigen

This radioactive material may be received, acquired, possessed, and used only by physicians, clinical laboratories, or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license, or a specific license, of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

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Name and Intended Use

CORAB is Abbott Laboratories' trademark for a qualitative and/or quantitative radioimmunoassay of Antibody to Hepatitis B Core Antigen (anti-HB_C) in serum or plasma.

Summary and Biological Principles of the Procedure

The CORAB test is a competitive radioimmunoassay in which nonradioactive anti-HB_C from serum or plasma competes with a constant amount of anti-HB_C ^{125}I (Human) for binding sites on beads coated with Hepatitis B Core Antigen (Human)[1]. Thus, the proportion of radioactive anti-HB_C bound to the bead is inversely proportional to the concentration of anti-HB_C in the test specimen.

After an incubation period, the bead is washed and the radioactive anti-HB_C bound to it is counted in a gamma scintillation well counter. Thus, within limits, the greater the amount of anti-HB_C in the specimen, the lower the count rate of the bead.

Explanation of the Test

After the incubation of specimens (or controls) and anti-HB_C ^{125}I (Human) with the beads is completed, and the beads have been washed and counted, the count rates of the unknowns are compared with a cutoff value (one-half of the sum of the negative control mean and the positive control mean). If the count rate of the unknown is lower than the cutoff value, the specimen is considered reactive for anti-HB_C. Specimens which are repeatably reactive by the CORAB test are considered positive by the criteria established for this test. Specimens which produce count rates greater than the cut-off value are considered negative for anti-HB_C and need not be tested further.

Reagents

1. Negative Control, CORAB (Recalcified human plasma, nonreactive for anti-HB_C and HB_SAg). Preservative: 0.1% Sodium Azide
2. Positive Control, CORAB (Recalcified human plasma, positive for anti-HB_C and anti-HB_S, nonreactive for HB_SAg). Negative Control, CORAB, is used to adjust the titer to 1:200 ± 2 $\log_2$ dilutions. Preservative: 0.1% Sodium Azide
3. Antibody to Hepatitis B Core Antigen ^{125}I (Human) in 0.05 *M* TRIS-0.04 *M* EDTA Buffer containing 50% Fetal Calf Serum and 2% recalcified normal plasma, human Radioactivity: 7.7 microcuries or less/ml. Preservative: 0.1% Sodium Azide.
4. Hepatitis B Core Antigen (Human) Coated Beads

Warnings or Precautions for Users

1. For In Vitro Diagnostic Use.
2. Do not use kit components beyond the expiration date.
3. Do not mix materials from different master lots.
4. When opening and removing aliquots from reagent vials, care should be taken to avoid microbial contamination of reagents.
5. Avoid unnecessary exposure to strong light during incubation or storage.
6. Handle all CORAB materials as though capable of transmitting hepatitis.
7. Handling should preclude any pipetting by mouth.
8. There should be no smoking or eating where radioactive or antigen-containing materials are being handled.
9. Hands should be covered with rubber gloves during, and thoroughly washed after, handling of radioactive materials.
10. Spills should be wiped up quickly and thoroughly with a 5% sodium hypochlorite solution and contaminated materials added to radioactive waste matter.
11. The specimens found to be reactive by the CORAB test and all materials used to perform the test should be disposed of as if they contained the infectious agent of viral hepatitis. The preferred method of disposal is autoclaving for a minimum of one hour at 121.5°C.
12. Liquid wastes may be mixed with sodium hypochlorite in volumes such that the final mixture contains 2.5% sodium hypochlorite.* Allow 30 minutes for decontamination to be completed. If the liquid waste contains radioactive material, this procedure should be performed in a well ventilated area.
13. Certain small quantities of ^{125}I liquid waste may be disposed of through a selected sink drain. Refer to the appropriate regulations applicable to your laboratory.
14. Holders of NRC Form 483 may dispose of solid waste by conventional means, after removing labeling. NRC Form 313 license holders should refer to Title 10, Code of Federal Regulations, Part 20. Licensees in Agreement States should refer to the appropriate regulations of their own state.

    Users holding a "Certificate of Registration", Form 483, or equivalent licenses of an Agreement State, may hold no more than 200 microcuries of radioactive iodine at one time. A maximum of two 100 test CORAB kits can be accommodated within this limit if these are the *only* sources of radiation on the premises.
15. This product contains sodium azide as a preservative. Sodium azide has been reported to form lead or copper azide in laboratory plumbing. These azides may explode on percussion, such as hammering.

    To prevent formation of lead or copper azide, flush drains thoroughly with water after disposing of solutions containing sodium azide.

    To remove contamination from old drains suspected of azide accumulation, the National Institute for Occupational Safety and Health recommends the following: (1) siphon liquid from trap using a rubber or plastic hose (2) fill with 10% sodium hydroxide solution (3) allow to stand 16 hours, and (4) flush well with water.

*Cossart, Y., Epidemiology of Serum Hepatitis, Brit. Med. Bull. 28:156, 1972. Snydman, D. R., Bryan, J. A., and Dixon, R. E., Prevention of Nosocomial Viral Hepatitis, Type B (Hepatitis B), Ann. Int. Med. 83:838, 1975.

Storage Instructions

1. Store all reagents at 2° to 8°C.
2. All reagents must be brought to room temperature for use.
3. The user shall store the radioactive material until used in the original shipping container or in a container providing equivalent radiation protection, including a refrigerator properly marked with a radiation hazard sign.

Specimen Collection and Preparation

1. All procedures of the CORAB test may be performed on human serum or plasma.
2. If specimens are to be stored, they should be refrigerated at 2° to 8°C or frozen. Sodium azide to a final concentration of 0.1% w/v may be added to retard biological growth. If specimens are to be shipped, they should be packed in compliance with Federal regulations covering the transportation of etiologic agents.
3. If desired, a specimen found positive by the screening procedure may be quantitatively assayed by serially diluting it in a plasma or serum which has been found negative by the criteria of the CORAB test.
4. Specimens containing copious amounts of precipitate may give inconsistent test results. To prevent this problem, such specimens should be clarified prior to assaying.

Procedure

Materials Provided

No. 5649, CORAB Kit (100 tests)

Kit contains:

- **4 Tubes (25 beads each) Hepatitis B Core Antigen (Human) Coated Beads and one dispensing tip.**
- **10 Vials (1 ml each) Antibody to Hepatitis B Core Antigen ^{125}I (Human). Radioactivity: 7.7 μCi or less/ml. Preservative: 0.1% Sodium Azide.**
- **1 Vial (3 ml) Negative Control, CORAB. (Recalcified human plasma, nonreactive for anti-HB_C and HB_SAg). Preservative: 0.1% Sodium Azide.**
- **1 Vial (3 ml) Positive Control, CORAB. (Recalcified human plasma, positive for anti-HB_C and anti-HB_S, nonreactive for HB_SAg). Titer 1:200 ± 2 $\log_2$ dilutions. Preservative: 0.1% Sodium Azide.**

Units of the above reagent kit are shipped in accordance with customer order.

An optimum combination of the following accessories is provided for performance of the tests ordered.

- **Reaction Trays (20 wells per tray)**
- **Cover Sealers (tear along perforation for use with 20 well trays)**
- **Tube Identification Inserts**
- **Assay Tubes with identifying cartons (for transfer of beads from Reaction Trays)**

Materials Required but not Provided

1. **Precision pipettes or similar equipment to deliver 100 μl.**
2. **Device for delivery of rinse solution such as Cornwall syringe, Filamatic or equivalent.**
3. **An aspiration device for washing coated beads such as cannula, aspirator tip, Uniwash™ II or Pentawash® II with a vacuum source and a trap for retaining the aspirate.**
4. **A well-type gamma scintillation detector capable of efficiently counting ^{125}I.**

Accessory Products

Washing Devices:

- **No. 7693, Uniwash II, washes and aspirates one well in a reaction tray. Must be used with appropriate vacuum and dispensing source.**
- **No. 6118, Pentawash II, washes and aspirates 5 wells in a reaction tray at one time. Must be used with appropriate vacuum and dispensing source.**

Miscellaneous:

- **No. 6152, Vacuum Pump, for use with No. 7693, Uniwash II; No. 6118, Pentawash II or equivalent.**
- **No. 8969, Dispensing Pump, for dispensing rinse water for use with No. 7693, Uniwash II; No. 6118, Pentawash II or equivalent.**

TM — Trademark

Performance of Test for the Detection of anti-HB_C

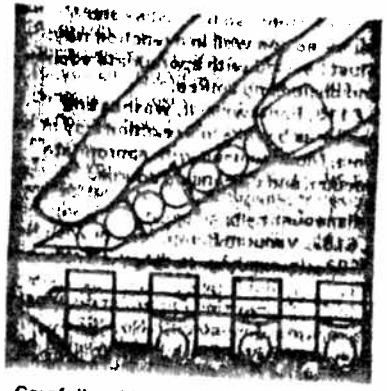
Specimens to be screened for anti-HB_C may be either serum or plasma and require no special preparation.

Specimens to be quantitatively assayed for anti-HB_C should be diluted in serum or plasma which does not contain anti-HB_S and which has a count rate within ± 25% of the CORAB Negative Control by the CORAB assay. It is suggested that appropriate serial dilutions be prepared and each dilution assayed by the following procedure.

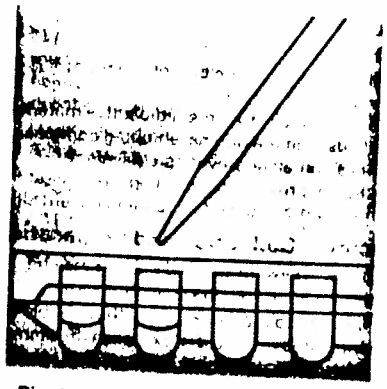
Five negative and five positive controls should be assayed with each run of unknowns. Ensure that all reaction trays containing controls and unknowns are subjected to the same process and incubation times. CAUTION: Use a clean pipette or disposable tip for each transfer to avoid cross-contamination.

NOTE: Once the assay has been started, all steps should be completed without interruption.

1. Bring all reagents to room temperature (20° to 30°C) before beginning the assay procedure. Mix gently before using.
2. Record (mark) position of each specimen (or control) in the reaction tray for proper identification.

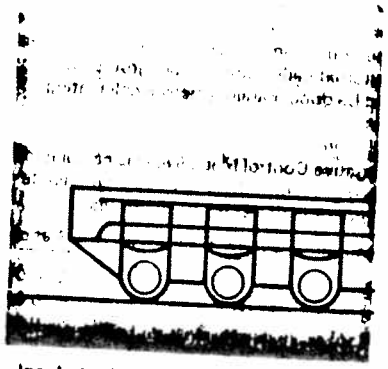


6. Carefully add one antigen coated bead to each well. Remove dispensing tip cover and hold dispenser directly over top of well. Push down tip with index finger to release one bead per well.

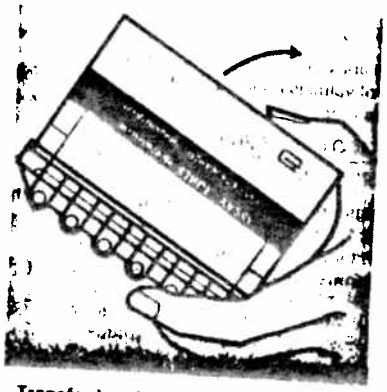


3. Pipette 100 µl Antibody to Hepatitis B Core Antigen ^{125}I (Human) into each designated well.
4. Pipette 100 µl of each specimen or control into its assigned well, using a separate pipette tip for each sample.
5. Tap the tray to mix the reagents, being careful not to splash or cross-contaminate the reaction mixtures.

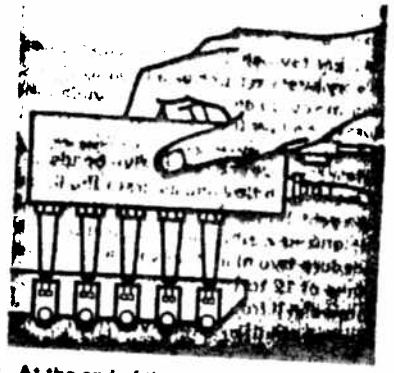
7. Cover the reaction trays with the cover sealers. Gently tap trays to ensure that each bead is covered with the reaction mixture. Be careful not to splash liquid onto the cover.



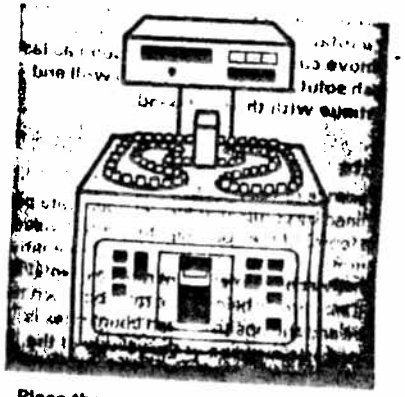
8. Incubate the trays on a level surface at room temperature for 18 to 22 hours.



10. Transfer beads from reaction wells to properly identified assay tubes. Align inverted rack of tubes over the reaction tray, press tubes tightly over wells, then invert tray and tubes together so that beads fall into corresponding tubes.



9. At the end of the incubation period, remove and discard the cover sealers. Aspirate the contents of the wells into a collection bottle for radioactive waste. Use a Pentawash II or equivalent device. See Wash Procedure Details, page 6. Wash the beads three times, each time with 4 to 5 ml of distilled or deionized water for a total rinse volume of 12 to 15 ml.



11. Place the assay tubes in a suitable well-type gamma scintillation counter and determine the net count rate for one minute. Although it is not critical that the counting be done immediately, it is preferable that it be done within 24 hours after the wash. All control samples and unknowns must be counted together.

Wash Procedure Details

1. **Washing five beads at one time.**

   Use a Pentawash II and an automatic delivery system (such as a Filamatic), a vacuum source and a trap for retaining the aspirate. Follow the directions accompanying the device: Lower the Pentawash II over a row of five beads in the incubation tray and aspirate the liquid. Rinse each bead with 4 to 5 ml of distilled or deionized water, then repeat the wash procedure two more times for a total rinse volume of 12 to 15 ml. Remove Pentawash II from beads as soon as the last wash solution is removed from wells, then proceed to the next row of beads.

2. **Washing one bead at a time.**

   Use a Cornwall Syringe delivery system (or equivalent) and a cannula or disposable pipette attached to a vacuum source. Place the cannula or pipette next to the bead and aspirate the liquid from the incubation well. Using care not to overflow the well, deliver 4 to 5 ml of distilled or deionized water to the well while simultaneously aspirating the wash solution. Repeat this wash two more times for a total rinse volume of 12 to 15 ml. Remove cannula or pipette as soon as last wash solution is removed from well and continue with the next bead.

Results

The presence or absence of anti-HB_C is determined by comparing the net count rate per minute (cpm) of the specimen to a cutoff value. This cutoff value is calculated from the negative and positive control net count rates as explained in the *Calculations* below. The ratio between the negative and the positive mean count rates is calculated as a measure of the validity of the test. The mean value for the negative control samples should be at least 5 times the positive control mean. If not, technique may be suspect and the run should be repeated.

Evaluation of the Test Validity

Count rates are to be recorded as *net* counts per minute. If the gamma scintillation counter does not automatically subtract background, this should be done manually before calculating results.

Calculations

1. Negative Control Mean ($NC\bar{x}$) is calculated by averaging the individual counts per minute (cpm) of the five negative controls.

Example. Negative Control No.	Net cpm
1	6,000
2	5,500
3	6,500
4	2,200
5	5,800
	Total 26,000

$$NC\bar{x} = \frac{26{,}000 \text{ cpm}}{5} = 5{,}200 \text{ cpm}$$

Elimination of Aberrant Values:

Discard those individual values in the negative control samples which fall outside of the range of 0.5 to 1.5 times the mean, i.e.,

0.5 x 5,200 cpm = 2,600 cpm
1.5 x 5,200 cpm = 7,800 cpm

In the above example, the fourth negative control value is rejected as aberrant. The revised $NC\bar{x}$ is then

$$\frac{26{,}000 \text{ cpm} - 2{,}200 \text{ cpm}}{5 - 1} = 5{,}950 \text{ cpm}$$

All negative control values should fall within the 0.5 to 1.5 $NC\bar{x}$. If more than one value is consistently outside the range, technique problems should be investigated.

2. Positive Control Mean ($PC\bar{x}$) is calculated by averaging the individual cpm of the five positive controls.

Example Positive Control No.	Net cpm
1	500
2	450
3	480
4	530
5	560
	Total 2,520

$$PC\bar{x} = \frac{2{,}520 \text{ cpm}}{5} = 504 \text{ cpm}$$

Eliminate individual aberrant values as for $NC\bar{x}$.

Determination of N/P Ratio

N/P Ratio is determined as follows:

$$\frac{NC\bar{x}}{PC\bar{x}} = \frac{5{,}950 \text{ cpm}}{504 \text{ cpm}} = 11.8$$

Interpretation:

While the actual cpm for the negative and positive controls will vary according to the age of reagents and the efficiency of the gamma counter used, the ratio of these values (N/P) is less variable. The N/P ratio must be greater than five (5) to ensure the validity of each run.

Evaluation of Specimen Results

Calculations:

The cutoff value is $NC\bar{x}$ + $PC\bar{x}$ divided by 2.

Example: $\frac{5{,}950 \text{ cpm} + 504 \text{ cpm}}{2} = 3{,}227 \text{ cpm}$

Note. If the gamma scintillation counter does not have automatic subtraction of background, the background cpm may be added directly to the cutoff value to eliminate the need of subtracting instrument background cpm from each specimen count rate.

Interpretation of Results:

- In the qualitative (screening) test:

  Specimens whose cpm rates are greater than the cutoff value are negative by the criteria of the CORAB test.

  Specimens whose cpm rates are equal to or lower than the cutoff value are considered reactive by the criteria of the CORAB test.

  Specimens which have been found repeatably reactive are positive for anti-HB_C by the criteria of the CORAB test.

- In the quantitative test:

  The titer of anti-HB_C in a positive specimen is determined by comparing the net count rate of appropriate specimen dilutions to the cutoff value.

  The specimen titer is defined as the dilution of that specimen which is most nearly equivalent to, but not greater than, the cutoff value.

Limitation of the Procedure

The CORAB test procedure is limited to the detection and quantitation of anti-HB_C in serum or plasma.

Expected Values

Specimens from three populations were screened for anti-HB_c with CORAB. As seen in the table, the incidence of anti-HB_c in the groups varied widely and is higher in paid donors than in the volunteers. Unselected patients in a city hospital also showed a high incidence of anti-HB_c.

Incidence of anti-HB_c in specimens from different populations as determined by CORAB

Population Description	Number Tested	anti-HB_c Positive	Percent Positive
Paid Donors	1017	77	7.6
Volunteer Donors	3913	87	2.2
Patients of a City Hospital	373	120	32.2

Specific Performance Characteristics

Precision

A reference panel of 20 specimens, 5 of which were within one two-fold dilution of the cutoff, was tested in duplicate on 4 or 5 separate days by 12 clinical laboratories using five master lots of CORAB. In a total of 2320 tests, there was 99.3% agreement. The mean cpm and the standard deviations about the means for these specimens are shown in the table below.

Reference Panel Data from Twelve Laboratories

Specimen Number	cpm		Standard Deviation (cpm)
101 (Negative Control)	6652	Negative (high cpm)	272
102	6242		1012
103	6160		266
104	6004		283
105	5871		1220
106	5554		948
107	5114		751
108	4404		197
Cutoff	3539		
109	3340	Positive (low cpm)	486
110	2918		387
111	2785		434
112	2542		168
113	2028		289
114	1716		139
115	1404		92
116	1305		185
117	733		127
118	600		92
119	531		69
120 (Positive Control)	525		69

Specificity

The presence of other hepatitis markers in a specimen has been found not to interfere with the CORAB assay for anti-HB_C.

Sensitivity

In the precision study, investigators obtained consistent results on all specimens that were within ± one two-fold dilution of the cutoff.

Bibliography

1. Overby, L. R., and Ling, C. M.: Radioimmune Assay for Anti-Core as Evidence for Exposure to Hepatitis B Virus. Rush-Presbyterian St. Luke's Med. Bull. 15:83-92, 1976.
2. Hoofnagle, J. H., Gerety, R. J., and Barker, L. F.: Antibody to Hepatitis B Virus Core in Man. Lancet ii:869-873, 1973.
3. Hoofnagle, J. H., Gerety, R. J., Ni L. and Barker, L. F.: Antibody to Hepatitis B Core Antigen: A Sensitive Indicator of HBV Replication. New Eng. J. Med. 290:1336-1340, 1974.
4. Lander, J. J., Gitnick, G. L., Gelb, L. H. and Aach, R. D.: Anticore Antibody Screening of Transfused Blood. Vox Sang. 34:77-80, 1978.
5. Szmuness, W., Hoofnagle, J. H., Stevens, C. E., and Prince, A. M.: Antibody Against the Hepatitis Type B Core Antigen. Am. J. Epidemiol. 104:256-262, 1976.

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