

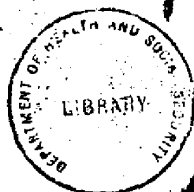
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NATIONAL HEALTH SERVICE



REVISED REPORT OF THE ADVISORY
GROUP ON TESTING FOR THE PRESENCE OF
AUSTRALIA (HEPATITIS ASSOCIATED)
ANTIGEN AND ITS ANTIBODY



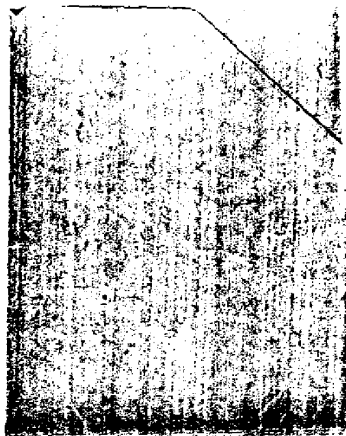
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CHAPTER 1

INTRODUCTION

1. A meeting convened by the Department on 20 July 1970 to discuss the problems of Australia (hepatitis-associated) antigen in relation to blood transfusion and associated matters, recommended that the Department should give any assistance it could "in the institution of testing blood donations for the presence of Australia (hepatitis-associated) antigen and its antibody".

2. In the light of this recommendation, we were appointed in September 1970 as an advisory group jointly by the Department of Health and Social Security, the Scottish Home and Health Department and the Welsh Office with the following terms of reference:—

"To advise the Health Departments on:—

- (i) the organisation of and responsibility for testing blood donations and other specimens of blood for Australia (hepatitis-associated) antigen and its antibody in the hospital service;
- (ii) the provision of reagents, choice of methods and whether, and if so, what kind of, training facilities are required;
- (iii) the scale of accommodation, staffing, equipment and other services necessary to implement the group's proposals."

Our members include Consultant Virologists, Directors of Regional Transfusion Centres and a Senior Technical Officer of the Public Health Laboratory Service.

3. We held our first meeting on 5 October 1970 and have met on six occasions. We have considered papers from a wide variety of sources at home and abroad including WHO, but have not felt it necessary specifically to invite evidence. We have considered it sufficient where necessary for individual members to make their own contacts with experts in a particular field. We have been assisted by the Departments' Supply Scientific and Technical Branch in procuring reagents for testing.

4. We have not included in this report the details of the methods of testing we recommend, or description of the detailed scientific background of the subject. The World Health Organisation Memorandum (1970) and the Report of a Symposium arranged by the International Society for Haematology (1970) and the papers to which they refer may be consulted by those responsible for testing for Australia (hepatitis associated) antigen and its antibody.

5. We have kept in touch with the Advisory Group under the Chairmanship of Lord Rosenheim which is examining the problem of infection risks in hospital renal failure units and our Chairman is a member of that group. We prepared for them a paper on blood and blood products. We have also been consulted by the Working Party on Health Hazards of the Central Pathology Committee on precautions to be observed when undertaking tests for Australia antigen, when handling "high-risk" specimens or when dealing with blood samples in hospital pathology laboratories. Our recommendations on Safety are at Chapter 6 below.

6. Knowledge of all aspects of Australia (hepatitis-associated) antigen is accumulating very rapidly. Our recommendations should therefore be regarded as interim ones. They are not exhaustive and may have to be modified in the light of new information.

CHAPTER 2

GENERAL PRINCIPALS OF TESTING

7. "Australia (hepatitis-associated) antigen" is the name used in WHO Memorandum (1970) for the antigen apparently associated with the infective agent(s) thought to be the cause of serum hepatitis. It is also known as "hepatitis-associated antigen" (abbreviation "HAA"), the "SH antigen", "Australia-SH antigen", "Au/SH", or "hepatitis antigen". We use the name "Australia (hepatitis-associated) antigen" or its shortened form "Australia antigen" in this report. The association between the antigen and serum hepatitis, commonly accepted as the most frequent form of hepatitis observed following the injection of blood and blood products, is well-established and the antigen can now be detected by a variety of laboratory tests. In particular, serological tests are available, one of the simpler and less sensitive of which detects the antigen in approximately 1 to 2 per 1,000 volunteer blood donors in Great Britain being tested for the first time. Australia antigen appears not to be associated with infectious hepatitis which may also be transmitted by blood and blood products. The case incidence of icteric hepatitis after transfusion of whole blood in a survey in 1954 was observed to be 0.2 per cent (Medical Research Council 1954); a survey, in progress, being conducted by an MRC Working Party, suggests that the case incidence of hepatitis, both icteric and anicteric, may be of the order of 4 to 5 per cent.

8. Although the hepatitis agent may be less widely dispersed in the U.K. than in some other countries, the institution of testing blood donations for Australia antigen should reduce the incidence of serum hepatitis, which is the most serious complication of transfusion, and so avoid suffering and disablement and even death. It would also lighten the load on the NHS. Estimates of the reduction vary but it should be about 25 per cent of the present incidence (WHO Memorandum 1970).

9. *We therefore recommend the institution of routine testing of all blood donations for Australia antigen and its antibody.*

10. Although the association between serum hepatitis and the injection of blood or blood products containing Australia (hepatitis associated) antigen is clear, the hazard from materials containing antibody—in the apparent absence of antigen—is less well documented. Our recommendation to test for both antigen and antibody by the techniques of immunodiffusion (ID), immunoelectroosmophoresis (IEOP) and complement fixation (CF) would, in the case of antibody, detect individuals with relatively large amounts. The recommended inclusion of tests for antibody by these techniques, and exclusion of donors found by them to be antibody positive, are made on the general grounds that the antibody indicates a past, or conceivably continuing, infection and that the exclusion of such donors is logically in line with present policies which exclude potential donors with a clinical history of hepatitis. Recent work, however, suggests that a proportion of the healthy population, negative for antigen and antibody by ID, IEOP or CF, may show antibody by highly sensitive techniques such as radioimmunoassay. So far as we are aware, the significance of such reactors as sources of infection is unassessed at the present time, and our recommendation about donors found to be antibody positive does not apply to them. It should be noted that, with regard to tests generally, positive results, either for antigen or antibody, may be transient and may not be confirmed, either in the same or in different laboratories, on samples taken from the same individual at, say, two or

three week intervals. Antigen/antibody complexes occur, and either antigen or antibody may be detected if one or the other is present in excess at the time of the tests. Theoretically, however, if the antigen and antibody were present in exact balance, neither might be detected serologically, at least by immunodiffusion, though the complexes might be detectable by electronmicroscopy.

11. The main source of available experience in respect of British blood donors is that of the Glasgow and West of Scotland Transfusion Centre where 13,950 donations tested by immunodiffusion revealed 17 cases with antigen (1 in 820) and one with antibody and a further 105,724 donations tested by immunoelectrosmophoresis yielded 86 with antigen (1 in 1,229) and 67 with antibody (1 in 1,578). Retesting by immunoelectrosmophoresis of a selected 5,574 of those tested by immunodiffusion yielded one further antigen-positive donation but an additional 12 antibody positive donations were detected. Regional Transfusion Centre, Sheffield, tested 44,166, by IEOP and found 1:1,162 antigen-positive and 1:2,324 antibody-positive. The removal of a proportion of donors of this order from donor panels would not seriously affect their size.

12. We recommend that blood donations should be tested in Regional Transfusion Centres rather than elsewhere because the results of the testing are usually needed within 24 hours of collection of the blood; it would thus not be practicable for these tests to be done in other laboratories on behalf of Regional Transfusion Centres (RTCs). In principle, we regard it as undesirable that the antigen and antibodies required for testing should be deliberately introduced into hospital pathological laboratories, except where there is a consultant virologist on the staff (see Appendix 2, Paragraph 2). In hospitals which organise donor panels and which do not have a consultant virologist, we recommend that specimens of locally collected donations be sent to the RTC or PHLS for testing. Where it is not possible to complete testing before a donation is issued, the clinician should be told that the donation has not been tested. This practice is similar to that followed for many years regarding donations transfused before syphilis-testing has been completed.

13. We also make recommendations about the reference centres, which should undertake any tests other than the routine tests on blood donations (Chapter 8).

CHAPTER 3

METHODS OF TESTING

14. A number of methods are available for detecting Australia antigen and antibody; others are being developed. Those at present used are therefore subject to change. Furthermore, appropriate reference preparations of antibody and antigen are not yet available and supplies both of antibody and antigen for testing are still limited. It is stressed that a negative result for antigen or antibody, obtained by any of the methods at present suitable for routine use, does not necessarily imply absence of an infective agent or agents.

15. At present three methods of testing are suitable for large scale screening: immunodiffusion (ID), complement fixation (CF), and immunoelectrosmophoresis (IEOP). These methods were described in detail in WHO Memorandum (1970). It may be possible in the future to employ a haemagglutination method which is under investigation.

Immunodiffusion

16. This is the simplest method. Its advantages, besides its simplicity, are economy in the use of reagents, ease of recording results and relative safety, providing covered dishes are used instead of slides. Its disadvantages are that it detects fewer positives than the other methods mentioned below and that results cannot be read until after 18 hours and, in some variations of the test, until after several days. Some laboratories recommend routine staining to bring up weak reactions. Immunodiffusion is useful for demonstrating the specificity of an antigen or antibody found in routine screening of donations.

The Complement-Fixation Test

17. The micro-titre complement-fixation test is used widely for serological diagnosis. It provides a method of quantitation of antigens and antibodies. Its advantages are that it detects a greater number of positive specimens than ID or IEOP, that the method may lend itself to automation, and that tests for antigen or for antibody can be completed within two hours. Overnight fixation may be used but is not essential though, without it, the test is less sensitive. Its disadvantages are that initial testing at a number of dilutions is recommended and that the test is expensive in terms of antibody to Australia antigen.

Immunoelectrosmophoresis

18. Many modifications of the principle of electrosyneresis have been published and the technique of immunoelectroprecipitation is currently described under many different names. We have decided to adopt the term immunoelectrosmophoresis (IEOP). The principal advantages of this method are that it is rapid and practical for screening blood donations providing the optimal operating conditions are used. It is essential to select suitable antisera and control antigen and to determine their optimal working concentrations. The technique may be helpful for detecting antibody in some sera which display anticomplementary activity due to the presence of antigen-antibody complexes. The disadvantage is a lack of comparability between results obtained in different laboratories; for example, some reports state that the test will detect two to ten times as many positive reactions as ID but others state that the results obtained using either method are similar. Tests for antigen and antibody are carried out simultaneously. The conditions of the test must be carefully

controlled to avoid false negative reactions when very high titred antigens are used (this is more common when the test is arranged for high sensitivity) and to avoid false positives caused by the migration of the control antigen past the centre well to react with the control antibody. The technique uses more reagents than ID but is more economical than CF. Strong positive reactions are easy to read after two to three hours but some authorities recommend that the slides or plates should be washed and stained in order to detect weak reactions. This technique is less sensitive than CF.

Haemagglutination and Haemagglutination-Inhibition

19. The techniques of haemagglutination and haemagglutination-inhibition, which have recently been used for detecting Australia antigen and antibody, appear to offer the advantages of simplicity, sensitivity and rapidity and lend themselves to automation. Experience of these techniques for this purpose so far is limited.

Recommended Method

20. We consider that immunodiffusion, complement fixation and immunoelectroosmophoresis are satisfactory methods for detecting Australia antigen or antibody. RTCs should aim to use a test which as far as possible combines speed, simplicity and sensitivity. IEOP appears to us to be the method of choice at the present time. Nevertheless, Regional Transfusion Directors may wish to use ID or to develop CF and we would not wish to dissuade them. A start has been made on developing automated procedures using CF or haemagglutination. We consider that this work should be pressed ahead, due regard being had to the difficulty of disinfecting contaminated equipment. In reference laboratories any or all of the above methods, as well as electronmicroscopy, will probably be used.

CHAPTER 4

STAFF AND TRAINING

Staff

21. In any laboratory in which testing for the presence of Australia antigen and its antibody is performed, it is considered that a consultant should be responsible for the organisation and direction of the testing laboratory, in view of the importance and potential hazards of the work and because of the problems which may arise in dealing with donors, patients and others found to be antigen-positive.

22. The laboratory in which these tests are carried out should be in the immediate charge of someone in a senior grade, who should preferably have had experience in bacteriology or virology. If a medical laboratory technician is appointed to the post, a grade appropriate to work of special responsibility should be chosen.

23. Once committed to total screening, a Regional Transfusion Director should arrange for the testing of at least a proportion of donations in such a manner that recently collected blood is available for issue after testing. Screening of all blood donations will impose a considerable, but predictable, work load on a regional transfusion centre. At the present time it is difficult to foresee accurately the future work load of reference centres; some estimates are given in para 51. In either type of laboratory the number of staff must be sufficient not only to provide a regular and continuing service, but also to reduce the risk of accidents. Overloading and overcrowding tend to cause technical and clerical errors, and may give rise to added hazards to staff handling infective material.

24. In one RTC, over a period of six months, two medical laboratory technicians at a time have tested 350 to 450 donor sera daily on Mondays to Fridays, and about 100 on Saturday mornings. To allow for regular changes of duties and to provide for holidays and sickness, a total of four technicians, whose grades in this case were a Chief Technician I (i/c laboratory), a Senior Technician II, a Senior Technician I, and a Technician (basic grade), received the necessary training. In addition one laboratory assistant was employed two afternoons each week to prepare plates. Provided supervision is adequate, we see no objection to the employment of a laboratory assistant in the testing procedures. The results were read in succession by each of two persons. The person in charge of this special laboratory had been actively engaged on the work, and had ensured that the local code of practice, which relates to the handling, processing and disposal of donations found to be positive for Australia antigen or its antibody, had been observed. We consider that these arrangements and scale of staffing will also be suitable for other transfusion laboratories. A regional transfusion centre dealing with the number of specimens mentioned would therefore need to appoint two more qualified medical laboratory technicians. Since two of the four technicians trained in this work will always be working on duties appropriate to their grade in other laboratories of the centre, the remaining two could be found from within the centre staff. The services of a junior laboratory technician, laboratory assistant or both might also be required.

Training

25. The principles employed in the recommended methods are familiar to medical laboratory staff. However, *ad hoc* training in these testing

procedures is essential; it may otherwise be difficult to obtain satisfactory and reproducible test results for Australia antigen and its antibody. In addition, to attempt to introduce these methods by trial and error would waste valuable reagents.

26. We recommend therefore that staff from transfusion laboratories about to begin work in this field should be seconded for training to transfusion laboratories already engaged in testing for Australia antigen and its antibody. Consideration should be given to the organisation of training courses or "workshops". In this way staff who had gained experience in a regional transfusion laboratory could share their experience with colleagues from other regions, and could benefit from the help and advice given by the staff of the PHLS reference centre.

CHAPTER 5

ACCOMMODATION AND EQUIPMENT FOR TESTING

27. Although the adoption, in any laboratory dealing with specimens potentially infected with Australia antigen, of the principles and techniques used in microbiological laboratories for dealing with infective material will afford a measure of protection, we feel it desirable that testing for the presence of Australia antigen (or its antibody), because it necessitates the deliberate introduction of Australia antigen as a positive control, should only be undertaken in RTCs (for the reasons given in para 12), or in laboratories where there is a consultant virologist, and that either laboratory should be staffed in accordance with the recommendations in para 24. This recommendation could be modified if Australia antigen were prepared in a form which was, demonstrably, non-infective. Such is not at present the case (see para 49).

Regional Transfusion Centres

28. We have based our recommendations concerning space required for testing in RTCs on the following assumptions: (a) that the method to be used will be IEOP (the space needed for CF, if automated, would probably be similar, save that additional floor space to accommodate a 4°C refrigerator (8-10 cu. ft.: 0.3 cu. m.) would be needed if overnight fixation were employed); (b) that testing for Australia antigen and its antibody only will be carried out (testing for other agents may also be required in the future but requirements for these are not now predictable); (c) that testing for syphilis will be carried out elsewhere in the building. There should be a high degree of isolation between the testing laboratory and the remainder of the RTC building, but we do not consider that complete physical isolation in a separate building is necessary; such isolation would often be highly inconvenient.

Tissue Typing (see also Appendix 2, paragraph 7)

29. RTCs and other laboratories which undertake tissue typing and histocompatibility testing should ensure segregation of this work and take precautions against infection similar to those taken when testing blood donations for Australia antigen.

Preparative Work

30. For work such as the preparation of slides or plates for IEOP, no special precautions affecting accommodation requirements are needed; an area of 150 sq. ft. has, in practice, been found adequate to prepare for 400 tests/day; an increase of 50 per cent in area should suffice for 800 tests/day.

General Serology Laboratory

31. We envisage that the separation of each specimen into portions for blood grouping, syphilis testing and for tests for Australia antigen and antibody would be done in the general serology laboratory. Initially about one in one thousand of these sera, i.e. about one specimen every other day in most centres, is likely to be antigen positive. Some of the precautions recommended below for the testing laboratory, would be required here, including provision of wash-hand basins and of floors and benches which can be washed down with disinfectants. Centrifugation probably creates the main specific hazard and the area occupied by centrifuges, benches, floors and walls should be easy to clean (see also paragraph 42). It is not

considered that there will be any need for extra space in the general serological laboratory on the introduction of testing for Australia antigen.

Testing Laboratory

32. The actual testing for Australia antigen must be carried out in a separate laboratory. A room of 200 sq. ft. with a bench run of 16 ft. is adequate for 400 tests/day. For 800 tests/day 32 ft. of bench are required. The door(s) of the room should be lockable. A warning light over the door and warning notice on the door should be provided to prevent unauthorised entry. A small ante-room, where supplies of protective clothing, etc. (see paragraph 3 of appendix 2), are kept and ordinary outer clothing left, is desirable. The surface of the walls should be washable, e.g. painted with gloss paint (epoxy paints are resistant to a variety of disinfectants, including "Chlorox", and stand up well to scrubbing). The flooring should be waterproof and likewise resistant to disinfectants, e.g. asphalt, rubber sheet or vinyl sheet (see paragraph 43). If asphalt is laid it should be coved to the wall. One wash-hand basin per 400 tests/day should be provided. Electric power points at 4 ft. intervals along the bench are essential, and gas points at 8 ft. intervals are desirable. Preferably, an autoclave should also be provided and there should be easy access to an incinerator outside the laboratory.

Retention of Samples of Donations

33. It has been suggested that ideally centres should store for six months, at least at -25°C and preferably below -30°C , a sample of serum from every donation tested. If a recipient should develop hepatitis within this time then the original specimen would be available for further investigation. If $\frac{3}{4}$ -in. by 2 $\frac{1}{4}$ -in. tubes were used for these samples, stored in aluminium storage racks, a centre doing 400 tests/day would require 34 cu. ft. (1 cu. m.) of freezer capacity. To store so many samples would require expensive equipment, considerable space and possibly extra staff. We therefore recommend that arrangements to store samples on this scale should be made in one region so that the value of keeping them can be assessed.

Equipment

34. At Appendix 1 are lists of suggested equipment required in the testing laboratory. They are based on the assumption that about 400 tests per day will be carried out by IEOP. We recommend, in the interests of safety, that an RTC carry out its normal immunohaematological reference work on any sample from a known positive patient in the same room as the routine Australia antigen testing is done, and we have listed at Part D of Appendix 1 the additional equipment which we suggest should be provided in the laboratory for this reference work on known positive specimens.

Virus Reference Centres (see also Chapter 8)

35. The work in these laboratories will consist of the investigation by various techniques of specimens referred from all sources, and the space requirements will vary according to the type and volume of work undertaken.

CHAPTER 6

SAFETY IN LABORATORIES (*see also Appendix 2*)

36. Satisfactory information regarding the incidence of hepatitis among laboratory workers is lacking but available evidence suggests that the incidence is not high. We consider, however, that the risk to laboratory staff will be minimised by the adoption, whenever possible, of microbiological procedures of dealing with blood specimens, together with a warning system for "high risk" specimens which we define below. Labelling of specimens as "high risk" does not imply that other samples, not so labelled, are "safe", and merely indicates that they are known to come from potentially infective sources. Matters of particular importance in laboratories dealing with blood samples are mentioned below; these are treated in greater detail in the CPC Handbook on Safety in Laboratories.

37. All staff should be informed of the potential risks and of the need for care when handling specimens of blood or blood products and of the need to maintain a high standard of personal hygiene. Sufficient wash-hand basins and supplies of disposable towels should be provided. It is important to take especial care to avoid spilling blood, and if this happens the blood should be cleaned up thoroughly with swabs soaked in disinfectant (see para 43 below).

38. We recommend that smoking, eating, drinking, licking of labels and mouth pipetting should be banned in areas of laboratories where blood specimens or blood products are dealt with.

Receipt of Blood Specimens

39. Staff responsible for receiving, unpacking and recording the receipt of blood specimens should wear apron and gloves, e.g. disposable plastic gloves. Specimens should be carefully packed, separate from the request form. A British Standard giving guidance on specimen containers is in preparation, but in any case "flip top" containers should not be used. At the discretion of the Consultant in charge of the laboratory or after the sender has been consulted, any container which has leaked or been broken in transit, and the associated packing, should be placed in a further plastic bag which should be sealed and incinerated or autoclaved. Blood-stained request forms should be destroyed. If it is decided that the accompanying specimen should be examined, the form should first be copied.

"High Risk" Specimens

40. We recommend that blood specimens from the following categories of patient should be labelled as "high risk" specimens, when the specimen is collected:

- (i) patients in renal failure units (e.g. for chronic haemodialysis or for kidney transplants);
- (ii) patients suffering from diseases of the liver;
- (iii) patients with defective or altered immunological competence, e.g. leukaemia, or Downs syndrome;
- (iv) patients known to be positive for Australia antigen or antibody.

Although there is at present no conclusive evidence to suggest that faeces and urine from cases of serum hepatitis are infective, it would be wise to assume that such specimens from the above patients may contain the infective agent, particularly if the excreta are thought to contain blood.

Transmission of high risk specimens

41. Special arrangements should be devised for transmission of specimens from "high risk" patients. These should be placed in glass containers fitted with a rubber-lined screw cap. We suggest that containers be placed in plastic bags, which can be made from lay-flat plastic tubing, closed by di-electric heat sealing. Stapling should on no account be used. The accompanying request form must not be placed in the same plastic bag as the container. These specimens should only be opened by the staff who are to process them.

Centrifugation of blood specimens

42. We consider that if a tube breaks or, in the case of angle centrifuges, if the tubes are overfilled during the centrifugation of blood, plasma or serum which may contain Australia antigen, the generation of aerosols is a potential hazard. In general, the use of angle centrifuges is to be discouraged. Centrifuge tubes containing "high risk" specimens should at least be capped or plugged or the tubes may be placed inside larger screw-capped tubes. Super-speed vacuum centrifuges could be hazardous if the tube seal fails and as an added precaution some workers cover material undergoing fractionation in sucrose or other density gradients with a film of mineral oil. Some intermediate range, unrefrigerated centrifuges have a hole in the bowl from which a stream of air is expelled when the rotor is turning, which may disseminate an infective aerosol. Enclosure of centrifuges in laminar air flow cabinets has been suggested, but this will not be effective unless the air streams generated by the centrifuge are effectively contained within the cabinet. No commercially available cabinet appears to us to meet this specification. We consider that rotating windshields afford some measure of protection and have asked the Department to arrange that small laboratory centrifuges fitted with windshields are made available. Alternatively, larger models with sealed buckets could be used.

Disinfection

43. As the infective agent(s) causing hepatitis has not been isolated, the effects of disinfectants upon it cannot be examined, and our recommendations are based upon the known effects of disinfectants upon enteroviruses. We suggest that hypochlorite solution (e.g. Chlorox) is the disinfectant of choice when Australia antigen may be present. It is usually supplied as a 10 per cent solution containing 100,000 ppm available chlorine. For general disinfection it should be diluted 1:100 to give 1,000 ppm available chlorine. Where blood has been spilt, or for disinfecting of equipment soiled with blood, a 1:10 dilution to give 10,000 ppm available chlorine should be used. The available chlorine in a solution gradually diminishes; a hypochlorite solution should not be used unless it turns starch iodide paper dark blue (i.e. available chlorine is not less than 200 ppm). Glutaraldehyde (2 per cent) and warm formaldehyde gas are also effective. For disinfection of the fixed parts of centrifuges or aluminium rotor heads we recommend glutaraldehyde or formaldehyde gas, as hypochlorite solution may cause corrosion. Contaminated buckets and other removable parts (other than aluminium rotors) should be soaked in glutaraldehyde and then autoclaved. If hypochlorite solution is used to disinfect the hands, when these have been contaminated with blood, they must then be thoroughly washed with soap and water. If the hands have not been directly contaminated, thorough washing with soap and water before leaving the laboratory is adequate. Sudol and chlorhexidine (Hibitane) are not considered effective against the causative agent(s) of hepatitis.

Accidents

44. A full record of each incident in which exposure to the causative agent may have occurred, should be kept. This should include, at least, the full name of the member of staff involved, the date and time, the reference number of specimen to which the member of staff has been exposed, a brief description of the incident, any treatment given, and the names of witnesses. Such incidents should include events such as the following; the list is not exhaustive:

- (a) a cut or other skin penetration caused by any needle, instrument or equipment contaminated with blood, blood components, or body fluids;
- (b) the aspiration or ingestion of blood, blood components or body fluids;
- (c) splashing of blood, blood components or body fluids on to the face, particularly the lips or the eyes;
- (d) extensive splashing with blood, blood components or body fluids over large areas of unprotected body surface;
- (e) the contamination by blood, blood components or body fluids of a skin surface which is visibly broken, e.g. dermatitis or previous cuts, and which has not been covered by protective clothing.

Immunoglobulin in prophylaxis

45. It has not been shown that human normal immunoglobulin (*gamma-globulin*), as at present prepared, will prevent serum hepatitis and evidence for any attenuating effect is meagre and inconclusive. While we do not ourselves recommend that human normal immunoglobulin should be used to prevent or attenuate serum hepatitis, this is a matter for the clinical discretion of the consultant concerned. However, human normal immunoglobulin will prevent infectious hepatitis and should be available for use if exposure to this disease is suspected. Investigation of the preventive and therapeutic value of immunoglobulin separated from plasma containing antibody to Australia antigen is at an early stage. In preparing this form of immunoglobulin, plasma from individuals whose blood contains antibody will be needed. Although we recommend (see para 57) that the blood of donors shown to contain antibody should not be used clinically and that their names be removed from the panel, their services should be retained in connection with the preparation of this specific immunoglobulin.

CHAPTER 7**ANTIBODY AND ANTIGEN AS REAGENTS FOR TESTING**

46. Antibody may be obtained from within the NHS or from commercial sources. We have obtained samples of available commercial antisera and tested them by immunodiffusion, complement fixation and immunoelectroosmophoresis. In the light of these examinations we advised the Department to buy one litre of antibody to be distributed to RTCs as an initial supply with which to start testing for Australia antigen.

47. The experience of certain RTCs suggests that once testing has started, sufficient potent antisera will be found among donors and selected patients to allow transfusion centres to become independent of commercial sources. We also hope that it will be possible to supplement human antibody with antibody prepared in animals and we regard research in developing such sources of antibody as most important.

Standard preparations

48. We consider as essential the establishment of reference preparations of antibody and antigen, the use of which will permit the potency and specificity of reagents to be compared. We recommend that new reagents, whether they be antibody or antigen, should be examined and their potency and specificity compared with these reference preparations. We also recommend that confirmation should be sought from the Standards Laboratory, Central Public Health Laboratory, Colindale, before such reagents are used for routine testing.

Heat-treated preparations of antigen

49. One of us (D.S.D.) has shown if a one in two dilution of plasma containing antigen is heated for 10 hours at 60°C it is rendered unusable in IEOP but its ability to react with antibody in ID is apparently unimpaired. We recommend that attempts to render the antigen-containing plasma or serum non-infective, while leaving it in a condition in which it will react normally with antibody, should be encouraged and supported because of the obvious advantages of having a non-infective antigen as a control reagent. Meanwhile we feel that it cannot necessarily be assumed that antigen-containing plasma which has been heat-treated is non-infective and urge caution in the use of such reagents.

CHAPTER 8

REFERENCE CENTRES

50. We regard as an essential part of the institution of testing for Australia antigen, the establishment of reference centres, supported by The Virus Reference Laboratory, and we note that the Department has made a grant to the Public Health Laboratory Service Board for this purpose. These Centres would undertake diagnostic work for hospitals which do not have a consultant virologist, examine suspected positive samples referred from RTCs, undertake survey work, e.g. in renal failure units and hospitals for the mentally handicapped, assess and develop new scientific techniques and characterise preparations of antibody and antigen used for routine testing for Australia antigen and its antibody. They should be in a position to apply any of the methods of testing used by RTCs and other laboratories that may refer specimens for confirmation.

Regional Reference Centres in England and Wales

51. We estimate that about 3,000 suspected positive specimens per annum will be referred from the RTCs in England and Wales for investigation. One of us (Y.E.C.) has estimated that the number of specimens from renal failure units may exceed 30,000 per annum. It is not possible to estimate the number of specimens from other sources. This load indicates that at least one centre should be located in each provincial region. We understand that the PHLS will establish centres in the following cities, and their addresses are at Appendix 5:

Birmingham	Exeter	Newcastle
Bristol	Leeds	Oxford
Cambridge	Liverpool	Portsmouth
Cardiff	Manchester	Sheffield

The Virus Reference Laboratory, Colindale, will act for the Metropolitan Regions as their local centre as well as acting as the central reference laboratory.

Regional Reference Centres in Scotland

52. We recommend that in Scotland Regional Hospital Boards should arrange for the establishment in each region of a centre in an appropriate laboratory, under the direction of a consultant virologist. These Centres should offer a service similar to that offered by the PHLS regional reference centres. It is to be hoped that these Scottish reference centres will maintain close liaison with the Virus Reference Laboratory, Colindale.

Central Reference Work

53. We recommend that the Public Health Laboratory Service Board should be invited to arrange for three laboratories at the Central Public Health Laboratory at Colindale to be involved in work on Australia antigen; the Virus Reference Laboratory to undertake its normal function of investigating "difficult" specimens on request from regional reference centres as well as the work referred to at 51 above; the Standards Laboratory to prepare, store and distribute reference preparations of antibody and antigen, and undertake validation work on reagents; and the Epidemiological Research Laboratory to continue its work in this field and support local epidemiological work. We have discussed the importance of instituting a scheme of quality control surveys of the testing for Australia antigen and its antibody, and have referred this problem to the PHLS Working Party on Laboratory Diagnosis of Hepatitis.

54. These proposals should meet the need to undertake diagnostic and epidemiological work on Australia antigen while concentrating it on as few centres as possible in view of the expertise required and the possible hazards of the work. We hope that the PHLS and Scottish Communicable Disease Report will include a summary of the results of testing. We suggest that the Department should consider whether the arrangements for the statistical analysis of notifications of jaundice can be modified to distinguish infective from serum hepatitis. We regard as most important close collaboration and exchange of information between reference centres and RTCs. In particular, the reference centres should report to RTCs all cases of hepatitis (whether Australia-antigen positive or not) which are thought to have been caused by blood or blood products, so that the recipients of other donations from the donor(s) concerned can be followed up and so that the donor(s) can be re-examined for the presence of Australia antigen or its antibody.

CHAPTER 9

AUSTRALIA ANTIGEN OR ANTIBODY POSITIVE SUBJECTS

Definition

55. We have agreed that a "positive" subject should be defined as one in whose blood Australia antigen has been detected or its presence at some time inferred from *finding antibody to it by IEOP, CF or ID. Positive findings should be confirmed by a reference laboratory (see Chapter 8). The serum should preferably have shown a reaction of identity with a known positive serum in the micro double diffusion in agar gel (Ouchterlony) test in the original laboratory, but the antigen may have been detected by complement fixation or immunoelectrosmophoresis. For confirmation of a positive result a second sample of blood should be obtained from the subject or, in the case of a donor, a sample of plasma should be taken from the donation itself to ensure that no error in identification of the donor blood has occurred. The serum should be tested by at least two techniques in the reference laboratory. If the original laboratory has used only CF, this technique will also have to be used in the reference laboratory if the results of other tests are negative.*

Donors

56. A donor whose blood is positive on the first screening should be suspended from the panel, and the donation concerned should be destroyed, unless required for the preparation of reagents. His certificate booklet should not be endorsed with this information. Testing in laboratories must be of a high standard in order to avoid false positives, resulting in the unnecessary suspension of donors and in order to achieve the highest possible detection rate for true positives. It may be necessary to review the recommendation that the name of a donor initially found to be positive should be suspended from the donor panel, if it is found, in practice, that a high proportion of cases are not confirmed by the reference laboratory.

57. We recommend that where a positive test has been confirmed by the reference laboratory, the donor should be removed from the panel and a letter should be sent to him informing him of these facts. The letter would invite him to tell the Director of the RTC the name of his family doctor, and if he is willing to do this a letter should then be sent to the latter. In the light of the recommendations in paragraph 45, the letters in respect of an antibody-positive donor should be suitably amended. At Appendices 3 and 4 are our suggested drafts of the letters. The letter to the family doctor points out that the implications of a positive test, to the individual concerned, are not yet clear and suggests that liver function tests be undertaken, and advises reference to a consulting physician, who has an interest in diseases of the liver, should the results prove abnormal. We suggest that SAMOs might be invited to undertake the necessary consultations before the names of such physicians are put forward.

Staff

58. Epidemiological evidence of transfer of antigen within transfusion laboratories (other than by accidental parenteral injection) is meagre. Evidence of transfer of infection from staff to blood or blood products is clearly difficult to obtain and we know of none. There is some evidence of transfer of infection from blood to staff. The number of known cases of clinical hepatitis among the staff of NBTS is, however, small (in absolute

terms) but the case incidence in relation to the total numbers of staff is not known, so that a comparison with the national incidence of infective jaundice (a notifiable disease) is not possible.

59. There are clearly sections of a transfusion centre where an antigen-positive or antibody-positive person could work without danger of his contaminating blood or blood products. Blood and blood products are prepared in closed systems or using aseptic procedures so that, theoretically, they should not be contaminated even if an antigen-positive person assisted in their preparation. It is probable that an antigen-positive or antibody-positive person would be immune to the infective agent and could, therefore, safely work on Australia antigen testing.

60. We have given much thought to the question of testing staff for the presence of Australia antigen. The purposes of testing, not necessarily in order of importance, would be (a) to prevent the remote possibility of blood or blood products from being contaminated by an antigen-positive or antibody-positive member of staff, (b) to demonstrate the awareness of the RTC of the risks involved, (c) to obtain information about the appearance of Australia antigen and antibody in members of staff, which might be valuable in the investigation of cases of hepatitis among them and in the study of the epidemiology of the disease, (d) to monitor the effectiveness of any means taken to protect staff, and (e) to permit early institution of symptomatic treatment of any members of staff in whose blood antigen is found.

61. Although the available evidence appears to indicate that the risk of an antigen-positive subject, let alone an antibody-positive subject, contaminating blood or blood products is very slight, we have concluded for the reasons in paragraph 60 that we should recommend that all staff joining the Blood Transfusion Service should be tested on appointment for the presence of antigen or antibody as a condition of appointment and that they and existing staff should be offered tests, which they should be urged to accept, at intervals of three to six months thereafter.

We further recommend that, until more is known about the epidemiology of serum hepatitis, a member of staff found to be antigen-positive should not assist in the preparation, by an open process, of blood or blood products destined for clinical use until he becomes negative. A member of staff found to be antigen-positive should be referred to his family doctor.

CHAPTER 10

SUMMARY OF PRINCIPAL RECOMMENDATIONS

62. For the reasons already given we make the following recommendations:

- (i) the Regional Transfusion Centres should begin, at the earliest possible date, to test all blood donations for the presence of Australia (hepatitis-associated) antigen and its antibody (9, 12);
- (ii) RTCs should use, initially, an immunoelectrosmophoretic method of testing (20), and follow our recommendations regarding staffing (22, 24), safety precautions (36 *et seq*), accommodation (32) and equipment (34);
- (iii) staff in RTCs should be tested, initially as a condition of appointment and afterwards voluntarily, and those found to be Australia antigen-positive should not assist in the preparation by an open process of blood or blood products destined for clinical use until they become negative (61);
- (iv) a donor found to be antigen or antibody positive should not be allowed to continue as a donor of blood intended for clinical use (56), and he should be told so and invited to give permission for his GP to be informed (57);
- (v) in the hospital service, it is desirable that only laboratories having a consultant virologist on their staff should undertake testing for Australia antigen (12, 50);
- (vi) the Public Health Laboratory Service Board should be invited to undertake the reference work arising from this testing and to assume the responsibility for testing specimens from other sources within the National Health Service (50 *et seq*);
- (vii) the Public Health Laboratory Service Board should be invited to offer a service for assessing the suitability of reagents to be used in routine testing of blood donations and specimens from members of staff or from patients (48);
- (viii) the Public Health Laboratory Service Board should be invited to consider the need for epidemiological studies of hepatitis in relation to blood transfusion and hospitalisation, and of the Australia antigen carrier state (53, 54);
- (ix) the Public Health Laboratory Service Board should be invited to consider arranging training courses or workshops for staff of RTCs (26).

References

- International Society for Haematology (1970), *Vox. Sang.*, **19**, 193-416.
 Medical Research Council (1954), *Lancet* **1**, 1328.
 World Health Memorandum (1970), *Bull. Wld. Hlth. Org.*, **42**, 957-992.

APPENDIX 1

EQUIPMENT (see paragraph 34)

(A) Suggested list of equipment required to test 400 samples per day by immunoelectroosmophoresis.

	<i>Approx. Cost</i>
Power Packs	
0-300 volt 80 mA D.C. constant voltage, constant current power supplies.	£
4 units, each driving 2 tanks	265
Tanks	
Electrophoresis tanks and associated apparatus.	
9 units, 2 per power pack with one spare to permit rotation	300
Switches	
Switches giving polarity control with current controlled cut-out.	
9 units, one per tank	100
Reading Lamps	
General purpose reading lamps	
2 units	35
	<u>700</u>

(B) Consumable supplies include:—

Petri Dishes*Sterlin* square petri dishes.
£22 per carton of 500.**Agarose**

£26.40 per 200 grams.

(C) Other equipment needed for the laboratory.

Tape Recorder

Battery/mains portable cassette recorder with 10 metre extension lead, mini jack, to allow elimination of writing records in the testing laboratory. The tape recorder itself will be outside the testing laboratory ...

40

Extract Cabinet

Cabinets to PHLS specification and BS 3202C having air velocity of 100 ft./min., an absolute filter (GAA), discharge to outside, and negative pressure in trunking

175

Laboratory Chairs

Chairs, high and swivel,

2 units 20

Lockers

Full length, 12-in. × 12-in. cross section

3 units 15

Autoclaves

For example, Autoclave, automatic, self-contained, electrically heated, no permanent air bleeds or escape of steam to atmosphere:

size 8-in. (diam.) × 12-in. (length).

Available on Central Supply. Stock No. 1105 ... 109

Levelling Table

For use in preparing gels 20

379

(D) Equipment required for work on known Australia antigen positive specimens requiring blood grouping, tissue typing, etc.

	<i>Approx. Cost</i>
	£
Centrifuge	
Should be protected as far as possible against aerosol generation. Suitable models with 4 × 50 ml. or 4 × 250 ml. buckets are being developed	300
Freezer	
Front opening	155
Refrigerator	
General purpose laboratory refrigerator On Central Supply—non-medical item	50
Incubator	
37°C. Anhydric thermostatically controlled Available on Central Supply. Stock No. 0285 ...	99
Water Bath	
Universal water bath with lid and adjustable shelf ...	60
	<u>664</u>
Total non-recurring cost — Approx. £1,800	

APPENDIX 2

CODE OF PRACTICE FOR LABORATORIES UNDERTAKING TESTS FOR AUSTRALIA (HEPATITIS-ASSOCIATED) ANTIGEN AND ITS ANTIBODY**Introduction**

1. These precautions are applicable to areas of the laboratory in which tests for Australia (hepatitis-associated) antigen and its antibody are carried out. They are *additional to the precautions for handling blood specimens for other investigations recommended in the handbook on laboratory safety to be issued by the Central Pathology Committee.* They are recommendations and are not exhaustive. Those in charge may wish to adopt other means of protection, in these areas of the laboratory, which are at least as effective. No matter what safeguards are provided for or precautions taken on behalf of the laboratory worker, there are no substitutes for constant care on his part.

Accommodation

2. In Regional Transfusion Centres, tests for Australia antigen and its antibody *must be done only in a special laboratory, specifically reserved for this purpose.* This laboratory must be kept locked to prevent the entry of unauthorised staff. Elsewhere, tests for Australia antigen and its antibody should only be done in microbiology laboratories, under the direction of a consultant virologist. Any rooms used for this work shall be provided with a wash-hand basin and disposable paper towels. All bench surfaces should be wiped down with 1 per cent hypochlorite solution after each day's work.

Protective Clothing

3. Disposable plastic aprons, gowns, masks and gloves should be worn and used once only. *Non-disposable plastic protective eyeshields should also be worn.* A supply of these items should be kept in the laboratory. Those working in this laboratory should, on entering, take off the laboratory coats they normally wear in other parts of the unit, hang them on pegs specifically provided, and put on the protective clothing mentioned above. Before leaving the laboratory, on any occasion, all items of used disposable clothing should be removed and immediately placed in containers which should then be sealed and later incinerated or, if this is not possible, autoclaved. The normal laboratory coat should be put on before leaving the laboratory.

Sharp Instruments

4. The use in this laboratory of needles and other sharp-pointed instruments should, if possible, be avoided altogether. If this is not possible their use should be reduced to the absolute minimum. Pasteur pipettes should have their ends rounded by flaming and be plugged with cotton wool. Preferably, disposable plastic capillary tubing should be used. Used pasteur pipettes should never be laid on the bench.

Isolation

5. Staff working in this laboratory in RTCs should not undertake work in any other part of the unit (particularly as their work is almost certain to involve handling of therapeutic materials such as

blood, blood products or other intravenous preparations). They should therefore be assigned to work in the Australia antigen testing laboratory for a continuous period at a time.

Disposal of contaminated equipment and samples

6. After testing, the disposal of known positive or high risk samples, and accompanying contaminated glassware, etc., needs special attention. Hypochlorite solution is inactivated by the presence of serum or plasma and although autoclaving is a satisfactory method of sterilisation, adequate cleaning of glassware contaminated with coagulated protein is exceedingly difficult. The use of disposable equipment whenever possible is therefore recommended. Glassware, etc., should be soaked overnight in hypochlorite solution before autoclaving, or autoclaved immediately, and then destroyed. Items of equipment for which destruction is inappropriate, should be soaked overnight, in hypochlorite solution or glutaraldehyde, autoclaved if possible, and then cleaned in the normal way. A suitable way of disposing of specimens would be to aspirate them into a closed glass container which would then be disconnected (together with its connecting tubes) and sealed, placed in a metal container with some water in it (to safeguard against breakage), and autoclaved, after which the glass container and its contents would be discarded.

Other Laboratory Examinations

7. Any known antigen-positive specimens accepted by a Regional Transfusion Centre for testing (for example, because of cross-matching difficulties or as a potential reagent containing a rare antibody) should be examined in the specified laboratory so that the appropriate precautions can be strictly observed. This laboratory should therefore be equipped so that the normal immunohaematological methods can be applied. In Regional Transfusion Centres, undertaking tissue typing or histocompatibility testing, it is recommended that arrangements should be made to screen in the specified laboratory any specimens originating in haemodialysis or renal transplant units. Attention is also drawn to the desirability of screening in this laboratory specimens which originate from other high risk sources.

APPENDIX 3

SUGGESTED LETTER TO POSITIVE DONORS

Dear Mr./Mrs./Miss,

I am sure that, as a (regular) blood donor, you know that the Blood Transfusion Service cannot accept donations from people who have had jaundice. Jaundice is often due to hepatitis (an inflammation of the liver) and such donations might therefore transmit hepatitis to patients receiving the blood. We have always known that hepatitis may also occur in such mild form that it is not recognised; jaundice does not appear and the person may feel quite well or only slightly indisposed. Until recently there has been no way of detecting these persons, although it was realised that transfusion of their blood might cause hepatitis.

Tests have now been devised which will identify a person whose blood may transmit hepatitis, even though he may be unaware that he has ever had this disease. Your blood has recently been found to be positive by these tests. It is therefore possible that you may in the past have had an attack of hepatitis of which you were unaware, and for this reason we shall have to withdraw your name from our active panel of donors and you should not be a blood donor in the future.

I think we ought to let your own doctor know about this finding, and I should be glad if you would agree to our doing this. If you would kindly send me his name and address, I will then write to him. You should let your dentist know, or show him this letter, at your next visit.

We are grateful to you for all the help you have given this vital service in the past and are very sorry indeed that we can no longer call upon you. May I offer you my warmest thanks.

Yours sincerely,

APPENDIX 4

SUGGESTED LETTER TO G.P. FOLLOWING AGREEMENT BY DONOR

Dear Dr.

Your patient (name and address) is a blood donor and routine testing has shown that he/she has a positive Australia-antigen/antibody test at the present time. He/She has been told that tests have shown that in the past, unknown to him/her, he/she may have had an attack of hepatitis. He/She has agreed that we should give this information to you.

The implications for your patient of the results of this test are not entirely clear at the present time, but it is known that, if his/her blood were transfused, the recipient might develop hepatitis. He/She should not, therefore, be a blood donor in the future and has been told this. In addition, he/she has been asked to inform his/her dentist of these findings at the next visit. Perhaps you would be good enough to remind him/her of the need to do so.

May I suggest that it would be desirable to have liver function tests done as he/she may possibly be incubating disease, and that you should consult the pathologist at your local hospital about these tests. If a specimen of blood is sent to the laboratory it should be clearly marked "Australia antigen-positive sample" and be most carefully collected, handled and packed. If the liver function tests show any abnormalities suggesting some form of hepatitis, you might be interested to know that Dr. of Hospital, who is specially interested in diseases of the liver, is willing to see such individuals should you think it necessary to refer your patient to him/her, or is willing as a matter of interest, to follow-up all such patients, even though their liver function tests might be normal and to all intents and purposes they appear to be healthy individuals.

Yours sincerely,

APPENDIX 5

PHLS Reference Centres for Australia Antigen

Birmingham:

Dr. T. H. Flewett,
Regional Virus Laboratory,
East Birmingham Hospital,
Bordesley Green East,
Birmingham B9 5ST.

Bristol:

Dr. S. K. R. Clarke,
Public Health Laboratory,
Myrtle Road,
Kingsdowne,
Bristol BS2 8EL.

Cambridge:

Dr. J. Nagington,
Public Health Laboratory,
Tennis Court Road,
Cambridge CB2 1QR.

Cardiff:

Dr. A. D. Evans,
Public Health Laboratory,
Institute of Pathology,
3rd Floor,
Royal Infirmary,
Cardiff CF2 1SZ.

Exeter:

Dr. R. J. C. Hart,
Public Health Laboratory,
Church Lane,
Heavitree,
Exeter EX2 5AD.

Leeds:

Dr. M. H. Hambling,
Public Health Laboratory,
Bridle Path,
York Road,
Leeds LS15 7TR.

Liverpool:

Dr. G. C. Turner,
Public Health Laboratory,
126 Mount Pleasant,
Liverpool L3 5SU.

London:

Dr. Y. E. Cossart,
Central Public Health
Laboratory,
Virus Reference Laboratory,
Colindale Avenue,
London NW9 5HT.

Manchester:

Dr. D. M. Jones,
Public Health Laboratory,
Withington Hospital,
Manchester M20 8LR.

Newcastle:

Dr. J. H. Hale,
Public Health Laboratory,
Institute of Pathology,
General Hospital,
Westgate Road,
Newcastle upon Tyne
NE4 6BE.

Oxford:

Dr. F. O. MacCallum,
United Oxford Hospitals,
Radcliffe Infirmary,
Oxford OX2 6AH.

Portsmouth:

Dr. J. V. T. Gostling,
Public Health Laboratory,
St. Mary's General Hospital,
East Wing,
Milton Road,
Portsmouth PO3 6AQ.

Sheffield:

Dr. M. A. M. Wilson,
Public Health Laboratory,
Northern General Hospital,
Herries Road,
Sheffield S5 7AU.

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