**Using the laboratory : a handbook for medical practitioners / prepared on the advice of the Standing Medical Advisory Committee and the Central Pathology Committee.**

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# Using the laboratory

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A Handbook for Medical Practitioners
prepared on the advice of the
Standing Medical Advisory Committee
and the Central Pathology Committee

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## 1.0 Introduction

### 1.1 The Laboratory

The policy of the Department of Health and Social Security is to ensure that all general practitioners have open access to pathology departments. This policy is welcomed by pathologists, who are anxious to be as helpful as possible. Pathologists also provide a domiciliary consultant service, under the usual rules and these two facilities may enable a diagnosis to be reached and a patient kept at home instead of being sent into hospital for investigation.

Every effort is made by laboratories to give a full, speedy, accurate and helpful service, but success depends also on the co-operation of those using the service.

### 1.2 The Pathologist

The most satisfactory relationship between clinician (whether GP or specialist) and pathologist is one in which the clinician may

discuss with the pathologist difficult problems with which he requires assistance. The pathologist may then be able to suggest how his help may be most effective and what specimens are required ; he can also assist in assessing the significance of the results.

## 1.3 The Handbook

These notes indicate the wide range of help a laboratory can give but do not purport to guarantee the universal availability of any particular aspect. The tables of procedures and of normal ranges are only a guide, and as methods vary they should be checked against local usage and results.

It is hoped that pathologists and medical practitioners will take advantage of the loose leaf system and insert their own notes and amendments in this handbook to suit local conditions.

## 1.4 Collection of Specimens

It is important that specimens should be delivered as early as possible so that the work can be dealt with on the same day. If the doctor regards a specimen as particularly urgent, it is essential that he makes contact with the pathologist personally so that the relevant degree of priority can be given to the tests.

Increasing workloads and mechanisation have resulted in some investigations being carried out in batches at specified times of the day.

More complex investigations, eg. plasma protein bound iodine (PBI), serum vitamin $B_{12}$, are usually tested in batches only on certain days of the week. Consequently close liaison with the laboratory and knowledge of the local batch arrangements will minimise delays.

The collection of certain specimens, particularly those involving serial blood examinations or timed urine collections, may be better performed in the laboratory than in the patient's home, and arrangements for such tests should be made with the laboratory. This will also give the pathologist the opportunity of seeing the patient, and relevant clinical information from the practitioner will be of the greatest value in assessing the clinical problem and the significance of the laboratory findings.

After blood has been taken by venepuncture with a minimum of venous stasis and the needle removed from the syringe, the blood should be carefully and gently expelled—forcible ejection of blood will produce haemolysis. If the tube used contains anticoagulant this should be dissolved in the blood by inverting the stoppered tube slowly several times taking care not to contaminate the skin.

Only dry-sterilised or disposable syringes should be used for collection of blood or cerebrospinal fluid.

## 1.5 Supply of Syringes and Containers

General Practitioners can obtain supplies of 2ml and of 10ml sterile

disposable syringes from their local Executive Council. The needle available and most commonly used for venepuncture is the 21G × 1½ inch (0.8 × 40mm or No. 1).
Containers should be obtained from the laboratory concerned with the investigation.

**Ministry Colour Code for Containers**

| *Nature of Anticoagulant* | *Colour code of label* | *Sizes available* |
|---|---|---|
| None | White | 5 and 10ml |
| Potassium Oxalate | Blue | 2.5 and 5ml |
| Sodium Citrate | Mauve | 2.0 and 2.5ml |
| Fluoride-Oxalate | Yellow | 2.0ml |
| Potassium EDTA (Sequestrene) | Pink | 2.5 and 5ml |
| Lithium Heparin | Orange | 5 and 10ml |

Citrate can evaporate if kept for more than a short time, in which case results may be affected. Old containers should therefore be discarded.
Universal containers (20ml), 4, 50, 100 and 200ml glass containers and sputum containers, and plastic 24-hour urine containers are generally available through the hospital laboratory services.

Cellulose wadding and suitable cardboard boxes for postal transmission of the containers are also obtainable from the same source.
A standard sampling outfit with instruction card for cervical cytodiagnosis is also available.

## 1.6 Danger of Jaundice from Handling Blood or its Constituents

Anyone who handles blood or its constituents may become infected with the causal agent of either serum hepatitis or infective hepatitis. The risk is greatest if cuts, abrasions, or needle-pricks afford a possible entry for the virus.
To reduce this risk to its smallest, it is important to prevent blood or its constituents from contaminating the skin or mucous membranes, such as those of the eye, mouth, lips and nose. A contaminated eye should be washed thoroughly and at once with clean water. If any other part of the body is contaminated it should at once be washed thoroughly with soap and water. (Brit. Med.J. (1966) 1,997).

## 1.7 Some Sources of Error

Technical errors can arise in the collection, labelling, and processing of specimens and in the recording and transmission of results. If an erroneous result is suspected the problem should be discussed with the pathologist. This practice is welcomed by the pathologist, as it

not only enables the individual result to be rechecked, but also acts as an additional form of quality control of results.
The contamination of the needle or specimen with antiseptic can lead to haemolysis of the red cells. Tourniquet constriction of the arm with venous stasis will adversely affect many results, as will any undue delay between collection and examination of specimens.
Sources of error relating to particular investigations are detailed in 5.1.1—5.1.4.

## 1.8 Clinical Information on Request Forms

In cases presenting new diagnostic problems it is desirable that request forms should be completed with every relevant detail, as without full information it is impossible to examine a specimen adequately, or to report on it constructively. For instance, the date of onset of the illness, its nature, the date and time when the specimen was taken and details of any treatment given before the specimen was taken can be relevant to the types and interpretation of the investigations carried out.
Rapid inter-continental transport and increased immigration are making 'tropical' and parasitic diseases commoner in this country. Details of foreign travel and of the patient's ethnic origin should be given when pertinent, eg. investigation of anaemias, pyrexia of undetermined origin, etc.

### 1.8.1 *Patient Identification*

Full identification of the patient must be given. The home address is a further valuable point of identification and should be supplied. In maternity cases, when blood transfusion is contemplated, the serial number of the Blood Transfusion Service and address are an advantage, if known.

The date of birth is a better aid to recognition than age but at least the latter should be given.

## 1.9 General Transmission of Specimens

Outfits for the collection and transmission of routine specimens should be obtained from the laboratory. Specimens should be sent to the laboratory only in containers specially intended for the purpose and not in other receptacles such as discarded food containers. (See Section 1.5 for information on containers.) For the less commonly submitted specimens, advice on the best method of collection can be obtained from the laboratory staff. It is frequently desirable and on occasion even essential that the patient attends the laboratory for the collection of the specimen. (Sections 5.0 5.1 6.2).

Containers must be clearly labelled with the patient's full name and date and time of collection of the specimen and they must be firmly closed as leaks will not only invalidate results, but may provide a considerable hazard to the health of laboratory staff and others.

### 1.9.1 ***Postal Transmission of Specimens***

See Post Office Guide for full Regulations but, in general the observation of the following provisions will satisfy the Regulations. Pathological specimens may be sent by letter post, but *not* by parcel post provided that:

(A) Any liquid must be in a sealed receptacle, which must itself be in an approved cardboard box or tin, with sufficient absorbent material (eg. cellulose wadding, or absorbent cotton wool to prevent any leakage in the event of damage. (Section 1.5).

(B) The packet must be marked 'Fragile with Care' and 'Pathological Specimen'.

## 2.0 Medical Microbiology

### 2.0.1 *General Information on the Transmission and Storage of Bacteriological Specimens*

Specimens should be handed into the laboratory or to a recognised collecting centre so as to reach the laboratory as soon as possible after taking. It is not generally desirable to send specimens for culture through the post; when this is done postal regulations for pathological specimens must be observed. (Section 1.9.1).

If specimens are likely to arrive outside usual laboratory hours, special arrangements should be made with the pathologist. When, for any reason, a specimen cannot reach the laboratory on the day it is taken, it should be kept overnight in a refrigerator, or, if the specimen is a swab, it should be put into transport medium (obtainable from the laboratory) and kept at room temperature. All swabs from which it is hoped to grow the more delicate organisms such as gonococci should be sent in transport medium. If bacteriological examination is to be done, a specimen should be taken before

antibiotic therapy or chemotherapy is begun. When pus or other body fluid is available, it is always preferable to send an ample sample, if not the whole specimen, rather than a swab.

## 2.1 Antibiotics

Every effort should be made to obtain a suitable specimen for bacteriological examination before antibiotic treatment or chemotherapy is commenced. This is especially important in pyrexia of undetermined origin, or when there is an unusual mode of presentation of an illness.

Sometimes unsuitable antibiotics will check bacterial multiplication sufficiently to nullify further bacteriological investigation, although the infection is not eliminated and the patient becomes seriously ill, eg. in some cases of bacterial endocarditis and bacterial meningitis. In these circumstances pathologists welcome early consultation and are frequently able to assist with advice on therapy.

## 2.2 Collection of Bacteriological Specimens

2.2.1 ***Urine Specimens:*** mid-stream specimens should be taken directly into wide-mouthed sterile containers. In males, it is best to send the patient to the laboratory whenever practicable. In women, the assistance of a district nurse or of the hospital out-patient

department is usually needed. Delay of more than two hours in sending urine to the laboratory may invalidate results, as contaminants multiply and cells degenerate or disappear. It is better to keep a urine specimen in a refrigerator than to let it stand at room temperature for more than 2 hours. In some laboratories facilities are available for direct plating of urine specimens by general practitioners.

2.2.2 ***Swabs from Dry Areas:*** eg. skin, nose, are not satisfactory unless the swab is first moistened—sterile water or tap water will do in an emergency although bacteriological 'broth' is better.

2.2.3 ***Vaginal Swabs:*** should *always* be sent in transport medium (supplied by the laboratory) in which trichomonas and gonococci survive well. A second swab should be used to make a film on a glass slide which should then be well dried before despatch. For diagnosis of gonorrhoea in the female, a cervical swab taken with the aid of a vaginal speculum is necessary.

2.2.4 ***Sputum:*** patients should be instructed that true sputum and not saliva is what is wanted (ie. collected by a deep cough preferably in the morning before drinking, eating or teeth cleaning has taken place).

2.2.5 ***Faeces:*** should be sent in a container supplied by the laboratory. For some purposes a rectal swab may be adequate, but advice should be sought from the pathologist.

Stool specimens can readily be collected in an ordinary water closet if

after micturition and flushing of the pan, 6 pieces of toilet paper be placed on the surface of the water before defaecation. Ample faeces will be found floating on the paper and a specimen may be lifted out on a wooden spatula and placed in a clean receptacle provided by the laboratory.

The labelled specimen should be sent to the laboratory accompanied by a request form stating the purpose of the examination, eg. 'for inflammatory exudate', 'for evidence of malabsorption', 'for culture for Salmonella or Shigella', 'for amoebae and cysts' or merely 'for parasites'.

2.2.6 ***Eye Swabs:*** unless there is a profuse discharge, eye swabs are likely to be unsatisfactory. It is preferable to send the patient to the laboratory when the specimen can be taken with a loop and then plated immediately. If swabs have to be used, they should always be sent in transport medium (obtainable from the laboratory).

2.2.7 ***Pertussis:*** pernasal swabs, obtainable from the laboratory, should be used. When collecting, the swab must reach into the nasopharynx and the swab should be sent in transport medium. Failure to observe these precautions is a common cause of failure to isolate the organism.

2.2.8 ***Staphylococcal Carriers:*** take swabs from the perineum and groin as well as from the anterior nares.

2.2.9 ***Streptococcal Carriers:*** swab anterior nares as well as throat.

2.2.10 ***Blood Culture:*** the laboratory should be contacted re blood cultures and appropriate equipment be obtained so that blood can be taken before antibiotics are given.

## 2.3 Specimens for Microbiological Clearance

Many patients become temporary carriers of intestinal pathogens after an infection. The Medical Officer of Health will advise on the need to delay return to work or school pending the clearance of the pathogen from the faeces, particularly where the patient is a food handler or has care of young children, or is otherwise specially likely to be a danger to the community. Where uneradicated infection is a danger to the patient's health, eg. in urinary tract infection, post-treatment specimens should always be sent for examination.

## 2.4 Specimens for Mycoplasma Investigation

*Mycoplasma pneumoniae* is the infective agent of one form of *'atypical pneumonia'. Mycoplasmas* may also be responsible for some cases of puerperal infection and non-bacterial urethritis. The pathologist should be consulted before sending specimens for mycoplasmal investigation.

## 2.5 Specimens for Mycology Investigation

Most mycological specimens are concerned with the diagnosis of

infection by dermatophytes ; they usually consist of skin, nail, or hair from infected areas. These may be sent by post in envelopes. Each sample should be wrapped in a small piece of paper, preferably black, and appropriately labelled.

For other specimens such as sputum, pus, urine, blood, serum or biopsy material, standard methods of transmission are suitable. When delay in transmission of the specimen is inevitable, the pathologist should be consulted about methods for the prevention of bacterial growth.

## 2.6 Specimens for Virology Investigation

In general, individual viruses or groups of viruses, like bacteria, cause illnesses having characteristic clinical features. The clinicians submitting specimens for virological examinations should, therefore, have some idea of the viruses most likely to have caused the illness. Unless this is so, it is impossible to collect the correct specimens at the optimal time and impossible to ensure that these specimens are examined for the appropriate viruses. The information in section 2.7 is designed to assist the clinician. When in doubt, the pathologist should be consulted.

It is commonly useless to submit, without personal consultation with the pathologist, specimens from a patient with an obscure illness, weeks or months after the onset. Material for virus isolation should be

collected as early as possible in the illness. In many virus infections, eg. those of the respiratory tract, viruses can be demonstrated for only a few days after the onset of symptoms. Some viruses however are excreted for quite long periods. The pathologist can advise the clinician in individual cases.

2.6.1 ***Virus Isolation***

For isolating viruses from the alimentary tract, faeces sufficient to fill one-third to one-half of a sterile universal container are required ; no preservative should be added. From other sites, except in patients with suspected smallpox, swabs broken off into a bottle containing transport medium (obtainable from the laboratory) are suitable. Cerebro-spinal fluid should be collected in a sterile universal container.

*In cases of suspected smallpox, the Medical Officer of Health should be informed at once and the collection of specimens arranged in consultation with him. The pathologist will be glad to assist if required.*

Virus survival at room temperature is usually short, with certain well known exceptions. Specimens should, therefore, reach the laboratory as rapidly as possible. Transport of specimens in a vacuum flask containing ice in a securely closed plastic bag is suggested. If storage of specimens for longer than 24 hours is necessary, consult the laboratory staff.

2.6.2 ***Virus Isolation—Interpretation of Results***

The isolation of virus from the site of disease, especially from parts of the body that are normally sterile, such as blood and CSF, is of far greater significance than its isolation from the respiratory or intestinal tracts where the presence of virus is not necessarily of aetiological significance. A significant rise in the titre of antibody to a virus isolated from a patient adds weight to the conclusion that the virus may have been the cause of the illness. The pathologist will always be glad to help in the interpretation of results.

2.6.3 ***Serological Tests in Microbiology—Paired Sera***

It is almost always desirable and frequently essential for diagnosis, especially in virus disease, to demonstrate a significant rise in antibody titre. At least two specimens of clotted blood (paired sera) are therefore necessary. Specimens should be taken,

(1) As early as possible in the illness and certainly within five days of onset.
(2) In the third week of illness : sometimes an additional specimen taken late in convalescence is of value.

A full history should be supplied including date of onset of illness, details of animal contacts, residence or recent visits abroad etc. When Widal tests are required details of TAB inoculations should be given on the request form, otherwise it may be impossible to

interpret results. In glandular fever the serum aminotransferases are sometimes increased, and the Paul-Bunnell is often negative in the early stages of the illness.

Complement-fixation tests are routinely available to detect antibodies to the following viruses or groups of viruses : *influenza; mumps; psittacosis; lymphogranuloma venereum; adenovirus; respiratory syncytial virus; measles; herpes simplex; Mycoplasma pneumoniae* (Eaton agent) ; and the *rickettsia of Q fever.* Antibodies to other viruses usually have to be demonstrated by neutralisation tests which are practicable only when a virus has already been isolated from the patient or his contacts. Tests for rubella virus antibodies are available in certain laboratories.

## 2.7 **Notes on the Investigation of Certain Bacterial and Viral Diseases**

(See 2.1 re antibiotics)

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Actinomycosis | Send whole Specimen of pus. | |
| Adenovirus infections | Throat swab in viral transport medium[1] : paired sera[2] : conjunctival swab : faeces. | |
| Anthrax | Material from the local lesion. | |
| Bornholm disease | See 'Coxsackie virus infections'. | |
| Botulism | The suspected food ; vomit ; faeces. | Inform the *Medical Officer of Health* and the *pathologist* at once. |
| Bronchitis | Sputum. | |
| Brucella infections | Blood culture : serum. | Brucellin skin test antigen available ; should not be used before serum is taken. (vide 2.9). |

2.7 (*continued*)

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Cat-scratch fever | No laboratory tests. | Skin test antigen available (vide 2.9). |
| Chickenpox | Vesicle fluid[1]. | Consult the pathologist. If there is any likelihood of smallpox, inform the Medical Officer of Health at once. |
| Conjunctivitis (bacterial) | Swab in bacterial transport medium. | |
| Conjunctivitis (viral) | See 'Adenovirus infections', 'Herpes Simplex', 'Smallpox'. | |
| Cowpox | See 'Smallpox'. | |
| Coxsackie Virus infections | Faeces : throat swab in viral transport medium : CSF[1] : paired sera[2] | |
| Diarrhoea | Faeces. | Consult the pathologist in outbreaks. |

2.7 (*continued*)

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Diphtheria | Nose and throat swabs. | Consult the pathologist. Give details of recent antibiotic therapy. The Medical Officer of Health should be informed of a suspected case. |
| Dysentery (bacillary) | Faeces. | Consult the pathologist in outbreaks. |
| Dysentery (protozoal) | Faeces. | Consult the pathologist. |
| Eaton agent | See 'Mycoplasma' (Sections 2.4 2.7). | |
| Echovirus infections | Faeces : throat swab in viral transport medium : CSF[1] : paired sera[2]. | |

2.7 *(continued)*

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Enteric fever (typhoid and paratyphoid) | Blood culture : faeces : urine : serum for Widal test (10ml clotted blood. Code White). | Give details of TAB inoculations. Consult the pathologist. The Medical Officer of Health should be informed of a suspected case. |
| Food poisoning | Faeces : suspected foods : vomit. | Consult the pathologist. Inform the Medical Officer of Health. |
| Fungus, infections of skin, hair and nails. | Hair stumps : portions of skin or nail : pus smears (Section 2.5). | |
| Glandular fever | Serum (5ml clotted blood, Code White), for biochemistry and serology and also as anticoagulated blood (2.5ml, Code Pink) for haematology (Sections 6.1 2.6.3). Liver Function Tests can be useful. | |

2.7 (*continued*)

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Gonorrhoea | Pus smear : swabs in bacterial transport medium (Section 2.2.3). | |
| Hand, foot and mouth disease, herpangina | Throat swab and swabs from lesion in viral transport medium : faeces[1] : paired sera[2]. | |
| Herpes simplex | Vesicle fluid : swabs from lesions in viral transport medium[1] : paired sera[2]. | |
| Herpes zoster | See 'Chickenpox'. | |
| Hydatid disease | Serum. | Skin test antigen available. (vide 2.9). |
| Infective hepatitis | Serum (to exclude leptospirosis and glandular fever). Liver Function Tests. | |
| Infectious mononucleosis | See 'Glandular fever'. | |

2.7 (*continued*)

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Influenza | Throat swab in viral transport medium[1] : paired sera[2]. | Consult the pathologist. |
| Leptospiral infections | Blood in EDTA bottle Code Pink in the first week of disease : urine : paired sera[2]. Liver Function Tests can be useful. | Consult the pathologist. |
| Lymphogranuloma | Serum. | Frei skin test antigen available, but should not be used before serum is taken (vide 2.9). |
| Malaria | Blood films : thick and thin (Section 3.1.1). | |
| Measles | Throat swab in viral transport medium[1] : paired sera[2]. | Consult the pathologist. |
| Meningitis (bacterial) | CSF : blood for culture. | |

2.7 *(continued)*

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Meningitis (viral) | See 'Coxsackie', 'Echo', 'Mumps', 'Poliomyelitis' and 'Leptospiral infections'. | |
| Mumps | Paired sera[2] : Throat swab in viral transport medium[1]. | |
| Mycoplasma infections | Paired sera[2] : Throat swab in viral transport medium[1]. | Consult the pathologist (Section 2.4). |
| Orf (contagious pustular dermatitis) | See 'Smallpox'. | |
| Ornithosis | See 'Psittacosis'. | |
| Pertussis | See 'Whooping Cough'. | |
| Poliomyelitis | Faeces : throat swab in viral transport medium[1] : paired sera[2]. | The Medical Officer of Health should be informed of a suspected case. |
| Psittacosis | Paired sera[2] : sputum. | |

2.7 (*continued*)

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Puerperal fever | High vaginal swab : urine. | |
| Pyrexia of undetermined origin. | Blood for culture : serum : faeces and urine. Blood for haematology and chemistry. | Consult the pathologist. |
| Q fever | Paired sera[2] : blood during fever. | Consult the pathologist. |
| Rheumatic fever | Throat swab : serum for antistreptolysin 'O' test. | |
| Rubella | Throat swab in viral transport medium : paired sera[2]. | Consult the pathologist. |
| Smallpox[3], Vaccinia Cowpox and Orf | Scrapings of macules or papules : smears and fluid from vesicles : scabs : conjunctival swabs if appropriate : serum. | Consult the pathologist. *If smallpox is suspected inform the Medical Officer of Health at once.* |
| Syphilis | Serum (Sections 4.1 to 4.1.6.) | For suspected primary—consult the pathologist. |

2.7 (*continued*)

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Thrush | Swab from lesion. | |
| Tonsillitis | Nose and throat swabs. | Consult the pathologist. |
| Toxoplasmosis | Serum. | |
| Trichomoniasis | Vaginal swab in bacterial transport medium. Air dried smear. | |
| Tuberculosis | Sputum : urine (three consecutive early morning specimens). | For other kinds of specimen consult the pathologist. |
| Urethritis | Pus smear, swab in transport medium. (Section 2.2.3). | If non specific cause suspected (eg. Mycoplasma) consult the pathologist. |
| Vaccinia | See 'Smallpox'. | |
| Weil's disease | See 'Leptospiral infections'. | |

2.7 (*continued*)

| *Disease* | *Type of specimen* | *Notes* |
|---|---|---|
| Whooping cough | Pernasal swab in bacterial transport medium (Section 2.2.7). | Consult the pathologist. |
| Worms | Whole worm (segments of tape worm may be identifiable) : eggs of thread worms may be collected by pressing against the peri-anal area the sticky side of a piece of transparent pressure-sensitive tape of the cellophane type which is then stuck to a slide. For eggs of other worms : Faeces. | |

[1]See Section 2.6.1

[2]See Section 2.6.3

[3]See Department of Health and Social Security and Scottish Home and Health Department 1969. *Diagnosis of smallpox: medical memorandum.* London, HMSO

## 2.8 Supplies of Vaccines, Antisera, and Diagnostic Skin Reagents.

### 2.8.1 Vaccines and Antisera

| | |
|---|---|
| Smallpox vaccine | Issued to individual doctors by most Medical Officers of Health. |
| Human immunoglobulin (previously known as Gamma Globulin) | Issued to individual doctors by most Public Health Laboratories. (For uses—see Monthly Bulletin, Ministry of Health, 1967, Vol. 26, Page 160 and British National Formulary 1968). |
| Human Antivaccinial Immunoglobulin | Issued to individual doctors by the Birmingham, Bristol, Cambridge, Cardiff, Leeds, Liverpool, London (Colindale), Manchester, Newcastle, Oxford and Sheffield Public Health Laboratories. |
| Rabies Vaccine and Antiserum | Issued to individual doctors by the Cardiff, Liverpool, London (Colindale) and Newcastle Public Health Laboratories. |
| Typhus Vaccine | Issued to individual doctors by the Birmingham, Bristol, Cambridge, Cardiff, Exeter, Leeds, Liverpool, London (Colindale), Manchester, Newcastle, Oxford and Sheffield Public Health Laboratories. |

2.8 (*continued*)

| | |
|---|---|
| Certain other antisera and antitoxins | May be obtained from certain hospitals. (For list, see Department of Health and Social Security's Memo. HM. (70) 37.) |
| Diphtheria, pertussis, poliomyelitis, tetanus and measles vaccines | May be obtained from the Medical Officer of Health. |
| TAB and other vaccines antitoxins and antisera | May be obtained through the usual commercial channels. |
| Yellow fever vaccination | Is done only at centres designated by the Department of Health and Social Security. (See Ministry of Health and Department of Health for Scotland, 1967. Notice to Travellers. Obtainable from the Department of Health and Social Security, the Scottish Home and Health Department or most travel agents). |

Notes on the uses of immunological preparations are contained in the British National Formulary (1968).

## 2.8.2 **Diagnostic Skin Test Antigens**

| | | |
|---|---|---|
| Brucellin | (for undulant fever) | Issued to pathologists by the |
| Casoni | (for Hydatid disease) | Standards Laboratory for |
| Cat-scratch fever | | Serological Reagents |
| Frei | (for lymphogranuloma Venereum) | Central Public Health Laboratory<br>Colindale Avenue |
| Trichina | (for trichinosis) | London NW9 |

## 3.0 Laboratory Tests in Tropical Medicine

### 3.1 In Cases of Fever Suspected of being due to a Tropical Infection

3.1.1 Malaria should be suspected in any acutely febrile patient who has returned from the tropics within the past three months. *In such cases Influenza should not be diagnosed in the absence of a blood test for malaria.* Thick and thin blood films should be made during the pyrexial phase, preferably while the fever is rising.

Though the chances of finding the parasites during the afebrile phase are small, blood slides should be taken whenever the opportunity presents itself.

Two microscopic slides, a hypodermic needle and a pledget of cotton wool soaked in ether only are required. A prick is made in the pulp of the finger which is then squeezed until a large drop of blood exudes. This is transferred to the middle of a slide, and spread with the pricked finger to cover an area the size of a sixpence. The finger is then cleaned and a smaller drop of blood is expressed : this is placed

at the end of the second slide and is spread into a thin film by means of the edge of the first slide in the usual manner. Both films are dried in air and not by heat and are sent by the quickest route to the laboratory, with a request for immediate examination and report by telephone.

3.1.2 A sustained pyrexia may suggest a Salmonella infection and calls for a leucocyte count, daily blood culture for 3 days and a base-line Widal (agglutination) test. However, since malignant tertian malaria (due to *P. falciparum*) may simulate typhoid, thick and thin blood slides should also be made. Blood cultures and blood for a Widal test should be sent to the laboratory (Sections 2.6.3 and 2.7, enteric fever).

3.1.3 Any pyrexial illness in a patient from the tropics which remains undiagnosed by ordinary clinical and radiological methods, aided by the tests outlined above, calls for hospital investigation preferably in a tropical disease centre.

### 3.2 In Cases of Suspected Schistosomiasis

If schistosomiasis (bilharzia) is suspected, terminal specimens of urine should be collected over 24 hours. These should be pooled and sent to the laboratory : ordinary cleanliness is required but not bacterial sterility.

3.3 An unusual and persistent skin eruption which does not itch, in the

form of nodules, thickened patches or areas of hypopigmentation, may in rare cases be due to leprosy. If this diagnosis is suspected the local Medical Officer of Health may be asked to arrange a consultation with the nearest member of the panel of leprosy opinion.

**3.4** A filarial infection may be suggested by lymphoedema of a limb, transient patches of giant urticaria with a high eosinophilia, the passage of a small worm across the eyeball or an irritant papular rash over the lower limbs. A leucocyte count will reveal an eosinophilia. The patient should be referred to the nearest Tropical Diseases Unit.

**3.5** Three specialised Tropical Diseases Units exist in Great Britain ; they are :

The Hospital for Tropical Diseases
4 St Pancras Way, London NW1
Telephone 01-387 6441

The Liverpool School of Tropical Medicine
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## 4.0 Immunology and Serology

(See also 2.6.3)

Serological investigations require blood taken into a dry sterile container (Code White) and allowed to clot. The specimen should reach the laboratory the same day or after overnight refrigeration at 4°C. For postal transmission it is preferable that the serum first be separated from the clot with sterile precautions.

## 4.1 Laboratory Investigations for the Diagnosis of Syphilis

### 4.1.1 *Suspected Early Syphilitic Lesions*

The patient should be referred to the laboratory for examination of a specimen from the lesion by darkground microscopy for the presence of *Treponema pallidum*. No treatment, local or general, should be given before this test has been carried out, but the application of a moist saline dressing to the lesion may make it easier to collect the specimen.

Blood should also be taken for serological tests.

### 4.1.2 *Screening Tests for Syphilis*

Complement-fixation tests (Wassermann) and/or floculation tests (VDRL slide test, Kahn and Price's tests) are used as routine tests for screening purposes and for follow up after treatment. One of the tests will usually be done quantitatively. The antibody (reagin) detected by these tests first appears about 7-10 days after the appearance of the chancre, is regularly present in the secondary and latent stages of infection and in most, but not all, late cases. Reversal to negativity after treatment depends on the stage of the disease when this is given. In early syphilis it usually occurs in 3-6 months. Latent and late cases may take several years before these tests revert to negative and a proportion of such patients remain seropositive despite adequate treatment.

False positive reactions may occur with these tests, which must be regarded as screening procedures rather than diagnostic tests. Such false results are found most often in association with febrile infections after smallpox vaccination and in the autoimmune diseases. They may occur in apparently normal persons and in pregnancy.

If a positive result is obtained in the absence of *definite* clinical evidence of syphilis, the tests should be repeated and the laboratory consulted about the advisability of a verification test.

### 4.1.3 *Reiter Protein Complement—Fixation Test*

In this, an extract of a non-pathogenic treponeme is used as antigen ;

it detects a different antibody from that revealed by the screening tests. When both the RPC-FT and the screening tests are frankly positive, the probability of treponemal infection is very high indeed.

4.1.4 ***Verification Tests***

Treponemal Immobilisation and Absorbed Fluorescent Treponemal Antibody Tests.

In these, virulent *Treponema pallidum* is used as antigen and these tests are thought to be specific for treponemal disease.

They are performed at only a few specialised laboratories and are not used as routine tests or as tests of cure.

The two indications for their use are :

(1) Patients with positive tests for reagin but no signs or history of syphilis, ie. where the diagnosis is between latent syphilis and a biological false positive test for reagin.

(2) Patients with clinical signs suggestive of late syphilis but in whom tests for reagin are negative or equivocal.

Neither these tests, nor those mentioned previously will distinguish between infections due to syphilis and those due to yaws. When specimens are sent for these tests, the patient should not have been on treatment with antibiotics ; full clinical information should be given to help in the interpretation of results.

### 4.1.5 *Tests on Cerebro-spinal Fluid for the Diagnosis of Syphilis*

The routine investigations should comprise a cell count, total protein estimation, Pandy or other test for excess globulin, Lange colloidal gold curve, and Wassermann reaction. The cell count, which is the most delicate index of disease activity, must be carried out within an hour of the specimen being taken.

## 4.2 Pregnancy Diagnosis Tests on Urine

For pregnancy testing the use of animals has now been almost completely replaced by immunological methods of detection of chorionic gonadotrophin. It is advisable that these tests be carried out in pathology laboratories because it is essential that the estimations are properly controlled.

Immunological tests are carried out on early morning specimens of urine, which should be kept in a refrigerator if storage is necessary. A minimum of about 5ml of urine is required. Different immunological tests vary in sensitivity but in general if the woman is pregnant the test will be positive from about 12 days after the date of missed menstrual period until a few days after delivery or intrauterine death : occasionally false negative results may occur after about 120 days. The very sensitive tests may give a positive result at about 5 days. In a clinically suspected early pregnancy a negative result should always be confirmed by testing after a further two weeks.

In routine use the lowest possible incidence of false positive results is desirable, and methods are selected for this purpose—even though an occasional false negative may occur. However, in some cases only low levels of gonadotrophins are excreted (eg. ectopic pregnancy) and the Pathologist should be informed if this diagnosis is suspected so that a more sensitive test can be used.

Protein, blood, and bacteria can interfere with some tests, giving false positive results. Positive pregnancy tests are found also in women with hydatiform mole and chorionepithelioma. In men with teratoma or seminoma of the testis such tests are also positive and if these are suspected this should be indicated to the Pathologist.

### 4.3 Tests for Rheumatoid Factors

Two tests for the detection of serum rheumatoid factors are available in special departments : 5ml of blood in a dry sterile container (Code White) is required. Both of these tests depend upon demonstrating in the patient's serum the presence of antibodies against immunoglobulins.

#### 4.3.1 *Sheep Cell Agglutination Test (Rose-Waaler)*

This test detects antibodies against rabbit gamma globulin on sheep red cells and it is positive in 70% of patients with definite rheumatoid arthritis. It may also be positive in systemic lupus erythematosus. High titres are often found in the presence of rheumatoid nodules

and tend to be associated with progressive disease. A single positive test, especially if of low titre, is not diagnostic as it may occur in febrile non-rheumatoid states.

4.3.2 ***The Latex Fixation Test***

This is a less specific but more sensitive test. High titres are usually found in rheumatoid arthritis and may also be present in systemic lupus erythematosus and the other collagenoses as well as in a variety of other disorders including infective hepatitis, glandular fever, and chronic infections.

## 4.4 Anti-nuclear Factors (ANF)

This investigation is primarily to detect in the patient's serum the presence of antibodies against nucleoprotein. Its chief value is to exclude the diagnosis of lupus erythematosus in which it is almost always positive. Serum titres greater than 1 :100 are found in systemic lupus erythematosus, in such cases the LE cell test is usually positive (Section 6.1). A negative anti-nuclear factor test virtually excludes a diagnosis of lupus erythematosus. In rheumatoid arthritis ANF may be present but is usually in lower titre and has not been shown conclusively to be related to the clinical features or prognosis of this disease. ANF is found also in rare conditions such as lupoid hepatitis, scleroderma, and dermatomyositis. The serum from 5ml of blood (Code White) is needed.

## 5.0 **Chemical Pathology**

The most commonly required biochemical investigations are set out below. A number of other investigations may be available after discussion with the senior staff of the laboratory. (Section 5.2).
In the case of infants and children it is often not desirable or possible to obtain a sufficiently large venous specimen for the usual laboratory techniques. Many laboratories have available suitable micromethods and it may be necessary to send the child to the hospital for the collection of a suitable specimen. It is usually essential that the laboratory be consulted before doing so.

5.1 The following table shows the Standard Determinations which are carried out by most Chemical Pathology Laboratories and the Normal Values which are found on the constituents of blood. 'Normal Range' is the range within which results from 95% of healthy adults should fall.

5.1 (*continued*)

| *Investigation* | *Usually determined in* | *Specimen required* | | *Normal value* |
|---|---|---|---|---|
| | | *Volume* | *Colour code* | |
| Amylase (Diastase). | Serum | 5ml | White | Less than 180 Somogyi units Section 5.1.3 |
| Bicarbonate | see Electrolytes | | | |
| Bilirubin | Serum | 5ml | White | Total : Less than 1mg/100ml (The Conjugated or direct reacting fraction is less than 0.2mg/ 100ml Direct van den Bergh is Negative) |
| Calcium | Serum | 5ml | White | 8.5–10.5mg/ 100ml |
| Chloride | see Electrolytes | | | |

5.1 (*continued*)

| *Investigation* | *Usually determined in* | *Specimen required* | | *Normal value* |
|---|---|---|---|---|
| | | *Volume* | *Colour code* | |
| Cholesterol | Serum | 5ml | White | *Old men 200–350mg/100ml<br>Young men 160–280mg/100ml<br>Women 10 to 20mg higher. |
| Creatinine | Serum or Plasma | 5ml | White or Blue | Less than 1mg/100ml |
| Dehydrogenases (Lactated) | Serum | 5ml | White<br>Section 5.1.1 | Section 5.1.3 |
| Electrolytes | Plasma (Serum is sometimes used) | 10ml | Orange<br>Section 5.1.1 | Bicarbonate 20–31mEq/l.<br>Chloride 94–106mEq/l.<br>Potassium 3.8–5.0mEq/l.<br>Sodium 135–145mEq/l. |

5.1 (*continued*)

| *Investigation* | *Usually determined in* | *Specimen required* | | *Normal value* |
|---|---|---|---|---|
| | | *Volume* | *Colour code* | |
| Fibrinogen | See Proteins | | | |
| Glucose | See Sugar | | | |
| Iodine (Protein-Bound) | Serum | 10ml | White<br>Section 5.1.2 | 4–8μg/100ml<br>Consult the Pathologist |
| Iron | Serum | 10ml | White<br>Section 5.1.4 | *Iron 80–180μg/100ml in men or 60–160μg/100ml in women<br>Total Ironbinding capacity 250–350μg/100ml |
| Phosphatases (Alkaline) | Serum | 5ml | White | Children<br>Up to 20K–A units/100ml<br>Adults<br>Up to 13K–A units/100ml<br>(Section 5.1.3) |

5.1 (*continued*)

| *Investigation* | *Usually determined in* | *Specimen required* | | *Normal value* |
|---|---|---|---|---|
| | | *Volume* | *Colour code* | |
| Phosphatases (Total Acid) | Serum | 5ml | White Section 5.1.1 | Up to 3K–A units/100ml (Gutman) |
| Phosphate (inorganic) | Serum (Preferably from fasting patient) | 5ml | White or Yellow | *Children 4–6mg/100ml Adults 2.5–4.5mg/100ml |
| Potassium | See Electrolytes | | | |
| Protein including Electrophoresis | Serum | 10ml | White | *Total protein 6–8g/100ml *Albumin 4–6g/100ml *Globulin 1.5–2.5g/100ml |
| Protein (Fibrinogen) | Plasma | 5ml | Blue or Pink | 0.2–0.4g/100ml |

5.1 (*continued*)

| *Investigation* | *Usually determined in* | *Specimen required* | | *Normal value* |
|---|---|---|---|---|
| | | *Volume* | *Colour code* | |
| Salicylate | Serum or Plasma | 5ml | Any type except Mauve | Nil |
| Sodium | See Electrolytes | | | |
| Sugar | Blood | 2ml | Yellow Some laboratories will accept capillary blood | *Fasting 65–100mg/100ml Random Values 65–175mg/ 100ml |
| Thymol Flocculation | Serum | 5ml | White | Thymol flocculation. Negative. Thymol turbidity. less than 4 Maclagan units. |
| Transaminases | Serum | 5ml | White Section 5.1.1 | Section 5.1.3 |

5.1 (*continued*)

| *Investigation* | *Usually determined in* | *Specimen required* | | *Normal value* |
|---|---|---|---|---|
| | | *Volume* | *Colour code* | |
| **Urea** | Blood | 5ml | Any except Yellow | Up to 40mg/ 100ml<br>In pregnancy, and childhood lower values up to about 25mg/100ml<br>Old people up to 50mg/100ml |
| **Uric Acid** | Serum | 5ml | White | *Below 7mg/ 100ml in men or 6mg/100ml in women. |

5.1.1 If results are expressed as millimoles per litre (mmol/l) and not as milliequivalents per litre (mEq/l), they will be numerically identical for monovalent ions (bicarbonate, chloride, potassium, sodium).

5.1.2 Values marked * are particularly subject to variation with the method used. With enzymes the activity varies according to the method of measurement, and different units may be used. Lists of local normal ranges should be consulted, or discussed with senior laboratory staff.

5.1.3 Change of value even within the normal range may be significant in an individual patient.

5.1.4 The administration of oral iron in prescribed or patent medicines within 48 hours of the test will invalidate the result of the serum iron.

5.1.5 Numerous drugs, (eg. Iodides especially in cough mixtures, Enterovioform, Rybarex, and Floxedil), affect the protein bound iodine and the pathologist should be consulted. In particular the protein bound iodine is useless as a diagnostic test (a) if the patient has been treated with any simple form of iodine during the previous 6 months. (b) if the patient has had an excretion pyelogram or choleystogram during the previous 18 months. (c) if the patient has *ever* had a contrast myelogram.
It may be misleading: (d) during pregnancy (e) if the patient is taking contraceptive pills.

5.1.6 Blood must be sent to the laboratory immediately for these tests.

Quite short delays may cause erroneous values, especially in measuring inorganic phosphate and potassium and acid phosphatase.

5.1.7 When multiple tests are required, less blood is needed than for the sum of the individual tests. The laboratory can advise.

## 5.2 Special Tests in Chemical Pathology

The pathologist must be consulted before any special procedure is arranged or undertaken. It is best if the patient can be sent to the Laboratory. In case of need, other arrangements can usually be made. Laboratories are normally willing after consultation to undertake the following tests on suitable patients at the direct request of general practitioners. Availability will vary according to facilities of the laboratory.

Urea or creatinine clearance tests
Glucose tolerance tests
Certain liver function tests
Certain thyroid function tests
Certain pituitary or adrenal function tests
Certain gastro-intestinal function tests

In the case of some particularly complicated or expensive tests prior consultation with the clinical staff of the hospital concerned may be appropriate.

## 5.3 Chemical Analyses of Urine

Urine should be sent to the laboratory as soon as possible after collection if the following quantitative analyses are required.

| *Investigation* | *Specimen required (Sections 5.3.1 5.3.2)* | *Normal value per 24hr (Section 5.3.3)* |
|---|---|---|
| Amylase | 24hr urine specimen | 3,000 Somogyi units |
| Ascorbic Acid (Saturation test) | 8hr overnight sample | 0–80mg/8hr |
| Calcium | 24hr urine specimen | 100–300mg |
| Chloride | | 100–250mEq |
| Creatine | | 0–50mg |
| Creatinine | | 1.0–2.0g |
| Potassium | | 40–120mEq |
| Protein | | 10–50mg |
| Sodium | | 100–250mEq |
| Steroids, 17 hydroxy-cortico– | | 0–20mg (5mg less in females) |
| 17 oxo. (keto) | | 10–25mg (5mg less in females) |
| Sugar | | 25–250mg |

5.3.1 ***Preservatives:*** the local laboratory should be consulted as to whether it is necessary to add a preservative to the urine for any particular test.

5.3.2 ***Procedure for collection of a 24 hour urine specimen***
On rising the patient empties the bladder. This specimen is discarded. All urine passed during the next 24 hours is collected into the special bottle. On rising the next morning the patient empties the bladder again, ie. 24 hours after the first specimen, and this final specimen is added to the 24 hour specimen. For most investigations collection from 8am to 8am is recommended. The times and dates of starting and finishing the collection must be clearly stated on the request form and the bottle.

5.3.3 Normal value is the range within which results from 95% of healthy adults fall. The excretion of many urinary constituents (eg. electrolytes, urea) depends markedly on the diet.

5.3.4 For qualitative testing (eg. Bence Jones Protein, 5-HIAA pregnancy tests, Section 4.2) an early morning specimen of 100ml will suffice.

## 5.4 Chemical Analyses of Faeces

5.4.1 Quantitative estimations (eg. fat) are best carried out on a three day collection. A 24 hour collection may give a rough guide but can be deceptive. The normal value for total fat is less than 5g per 24 hours based on a three-day collection.

5.4.2 ***Occult blood test:*** for the most sensitive tests specimens should be sent to the laboratory. Then, to eliminate false weak positives the patient should have been on a special meat free diet for the previous three days : a small portion (eg. 20g) from each of 3 morning specimens will be sufficient. (Section 2.2.5).

## 6.0 Haematology

### 6.0.1 *General Notes on Haematological Investigation*

In routine haematological practice it is often necessary to use general screening tests to avoid overloading the laboratory facilities. It is recognised that there will be clinically indicated cases when it is desirable to proceed initially with more elaborate tests, these should be referred to on the request form. The important screening tests are :

(a) Measurement of haemoglobin and examination of stained film.

(b) Measurement of ESR (Erythrocyte sedimentation rate).

These can be done on a single specimen of blood treated with potassium EDTA (Code Pink). Heparin sometimes causes agglutination of leucocytes.

The results of these screening tests will in many cases indicate a probable diagnosis and further tests to establish a more definite diagnosis can often be carried out on the same sample.

A list of drugs which the patient is receiving should always be recorded on the form in all cases of suspected anaemia, leucopenia or purpura because many drugs can produce these conditions.

## 6.1 **Haematology Tests, Specimens required** (Refer to 1.5 for colour code)

| *Investigation* | *Specimen required* | | *Normal value (see 6.1.1) and comments* |
|---|---|---|---|
| | *Volume* | *Colour code* | |
| Anti Human Globulin Test (Coombs) | 5ml | White | |
| Blood counts including Hb, PCV, RBC, WBC Reticulocytes, Platelets | 2.5ml | Pink | See separate tables |
| Blood Grouping and Cross Matching | 10ml* | White | |
| Erythrocyte Sedimentation Rate, (ESR) (Westergren) | 2.0ml | Mauve (2.0ml) | 1.6ml blood 0.4ml citrate– fill up to 2.0ml mark see separate table for normals |
| Glucose–6–Phosphate Dehydrogenase Deficiency | 2.5ml | Pink, Orange or Mauve | Consult Pathologist |
| Haemagglutinins | 5ml* | White | May be necessary to collect at 37°C Section 6.1.3 |
| Haemolysins | 5ml | White | Section 6.1.3 |

* Clotted Blood

6.1 (*continued*)

| *Investigation* | *Specimen required* | | *Normal value* (*see 6.1.1*) *and comments* |
|---|---|---|---|
| | *Volume* | *Colour code* | |
| Haemoglobinopathies | 2.5ml | Pink, Orange or Mauve | |
| LE Cell Test | 10ml | Defibrinated | Section 6.1.2 |
| Osmotic Fragility | 10ml | Orange | Defibrinated specimens also used Section 6.1.2 |
| Paul Bunnell | 5ml* | White | Consult Pathologist. Collect blood for WBC and differential count. |
| Prothrombin | 2.0ml | Mauve Section 6.3 | 1.8ml blood and 0.2ml citrate—fill up to 2.0ml mark |
| Serum Vitamin $B_{12}$ | 10ml* | White Section 6.1.4 | 150–1000 picogrammes/ml (pico—$=10^{-12}$) |
| Serum Folate | 5ml* | White Section 6.1.4 | Above 5 nanogrammes/ml Consult Pathologist (nano—$=10^{-9}$) |

* Clotted Blood

6.1.1 The normal values given (the range within which results from 95 per cent of healthy subjects fall) should be taken as a general indication only. In seeking guidance not only on the investigation of patients but in the interpretation of results, the pathologist should be consulted.

6.1.2 There are many methods for defibrinating blood. The simplest is to put 6-12 paperclips or glass beads in a *stoppered* universal container (Section 1.6), add 10-15ml of blood and invert at intervals of about 1 second for a minimum of 10 minutes.

6.1.3 Haemagglutinins and haemolysin titres may be affected by cooling the blood. It may be necessary on some occasions to collect and separate samples at 37°C.

6.1.4 Serum samples collected for vitamin $B_{12}$ or folate bio-assay must be sterile. Particular precautions should therefore be taken with skin cleansing and aseptic techniques.

## 6.2 Bleeding and Clotting Disorders

If these are suspected it is best for the patients to be investigated by the pathologist and the necessary samples taken at the laboratory.

## 6.3 Control of Anticoagulant Therapy (Prothrombin Time)

During anticoagulant therapy the control of dosage, usually a hospital clinic responsibility, is sometimes undertaken by the general practitioner but this should always be based on prothrombin time

determinations performed by the pathologist. The prothrombin test is done on blood collected by clean (ie. without tissue fluid) venepuncture which is then placed in a container containing citrate solution. The container should be checked to ensure that the citrate solution has not evaporated. The volume of blood required is marked on the container.

Containers with citrate solution as anticoagulant must be stored in a refrigerator.

## 6.4 **Haematology Normal Values** (Section 6.1.1)

### 6.4.1 ***Normal Erythrocyte and Platelet Values***

| | *Haemo-globin g/100ml* | *Erythrocytes millions per mm³* | *Packed cell volume (PCV or haematocrit value* |
|---|---|---|---|
| Men | 13.5–18.0 | 4.5–6.5 | 40–54% |
| Women | 11.5–16.5 | 3.9–5.6 | 35–47% |
| Infants (cord Blood) | 13.6–19.6 | 4.0–5.6 | 44–62% |
| Children, 1 year (mean)* | 11.2 | 4.5 | 35% |
| Children, 10 years (mean)* | 12.9 | 4.7 | 37.5% |

6.4.1 (*continued*)

| | |
|---|---|
| Mean corpuscular volume (MCV) | 76–96 $\mu m^3$ |
| Mean corpuscular haemoglobin (MCH) | 27–32 picogrammes (pg) (pico=$10^{-12}$) |
| Mean corpuscular haemoglobin concentration (MCHC) | 32–36% |
| Platelets | 150000–400000/$mm^3$ |
| Reticulocytes (adult) | 0.2–2% |

6.4.2 ***Normal Leucocyte Counts***

| | *Leucocytes per $mm^3$* |
|---|---|
| Adults | 4,000–11,000 |
| Infants at birth | 10,000–25,000 |
| Mothers after delivery | 10,000–25,000 |
| Pregnant women | up to 17,000 |
| Infants 1 year | 6,000–18,000 |
| Children 4–7 years* | 6,000–15,000 |
| Children 8–12 years* | 4,500–13,000 |

At all ages, recent vigorous exercise, either physiological or pathological such as an epileptic fit, causes a leucocytosis of up to 30,000 per $mm^3$ and return to normal may be delayed for 4-6 hours. A leucocytosis and raised ESR may also be found in a normal pregnant woman.

### 6.4.3 ***Normal Differential Leucocyte Count (adults)***

| | % | *Absolute (per $mm^3$)* |
|---|---|---|
| Neutrophils | 40–75 | 2,500–7,500 |
| Eosinophils | 1–6 | 40–440 |
| Basophils | below 1% | 0–100 |
| Lymphocytes | 20–45 | 1,500–3,500 |
| Monocytes | 2–10 | 200–800 |

***Infants and children***

The lymphocyte count steadily falls during infancy and childhood, from about 12,000 per $mm^3$ a week after birth to about 4,000 per $mm^3$ at 4 years and adult figures at 10 years or just before the onset of puberty.

***Error***

The range of unavoidable error in differential counts is very large. If eosinophil counts are considered important they should be specifically requested.

## 6.4.4 ***Normal Erythrocyte Sedimentation Rates***

The Westergren method is commonly used ; with this the normal range varies somewhat with age. The most acceptable figures are :

| *Age* | *Westergren method mm in 1st hour* | |
|---|---|---|
| | *men* | *women* |
| 18–30 | 7 | 10 |
| 31–40 | 8 | 11 |
| 41–50 | 10 | 13 |
| 51–60 | 12 | 18 |

Most of the figures given here are derived from Practical Haematology, by J.V. Dacie and S.M. Lewis, 4th edition, 1968, Churchill, London, and there are other published series.

*No satisfactory consistent figures of either optimal or normal values are known for infants or children. The estimation of the MCHC will usually detect iron deficiency in children, but thalassaemia and S/thal. disease in those of Mediterranean, and sickle-cell or S/C disease in those of African or West Indian origin, also give low values for the MCHC and such cases should be referred to the laboratory for further study.

## 6.5 Blood Groups and Transfusion

Knowledge of a patient's ABO and Rh groups is essential (1) to any doctor who is looking after a patient during her pregnancy and confinement and (2) when blood transfusion is required.

### 6.5.1 *Blood Groups and Pregnancy*

Haemolytic disease of the newborn may affect an infant if its blood group is incompatible with that of its mother. The disease most commonly occurs when an Rh-negative mother whose husband is Rh-positive, carries an Rh-positive child. The blood group antigen concerned in the majority of cases is the D antigen of the Rh Blood group system. The mother being Rh-negative, and therefore lacking the D antigen, may respond to the presence of the D antigen in her Rh-positive child by forming antibodies, which pass across the placenta into the foetus and cause red cell destruction from which all the features of haemolytic disease stem.

Although incompatibility involving the D antigen of the Rhesus blood group system is responsible for the majority of cases, the disease may also occur in the presence of foetal-maternal ABO incompatibility (most of the remaining cases) and incompatibility involving the other Rhesus antigens and those of other blood group systems.

Rh haemolytic disease, unlike that due to ABO in compatibility, very rarely occurs in first pregnancies unless the mother has been

sensitized by previous transfusion or undisclosed miscarriage or intramuscular injection of Rh-positive blood. (For a fuller discussion of haemolytic disease of the newborn see the pamphlet: Haemolytic Disease of the Newborn, Central Health Services Council, Ministry of Health).

6.5.2 ***Protection of Rh-negative mothers against sensitization to the D antigen***

Sensitization to the D antigen mainly occurs at the time of delivery when Rh-positive cells, carrying this antigen, may cross the placenta from the Rh-positive foetus into the mother's circulation. If anti-D immunoglobulin is given to the mother within a short time of delivery, the Rh-positive cells are removed from her circulation and sensitization is prevented in the very great majority of cases.

The pathologist or Director of the Regional Transfusion Centre will give you details of the availability of anti-D immunoglobulin and of arrangements for its supply.

6.5.3 ***First Blood Sample During Pregnancy***

The blood group is usually determined when pregnancy is diagnosed. This test and also the measurement of haemoglobin concentration and a syphilis test are performed routinely upon patients referred to antenatal clinics. The samples mentioned below should be taken from patients who are not referred to antenatal clinics. They should

be sent to the laboratory immediately, in order to avoid deterioration :

(a) For blood grouping and antibody testing and for syphilis testing : 10ml of blood in a dry sterile tube/tubes. (Code White)
(b) For haemoglobin measurement : 2 ml of blood collected into Potassium EDTA (Code Pink).

The sample must be clearly and carefully labelled so that it can be identified with the patient and associated with the form bearing the personal and clinical details. This information must be provided to guide the pathologist in selecting the blood grouping tests to be performed and must include :

(a) Expected date of delivery
(b) Parity
(c) A summary of the dates and outcome of previous pregnancies, particularly still-birth, jaundice, anaemia or foetal abnormality.
(d) Details of any transfusion or intramuscular injection of blood.
(e) Preferably it should also indicate whether tests have previously been made and give their laboratory reference numbers. (A standard form, NBTS 1, for recording these details is obtainable from pathological laboratories or regional transfusion centres).

The laboratory report returned to the doctor may indicate whether further tests are required during pregnancy and, if so, when. A green

blood group card (NBTS 3), to be given to the patient, is usually issued when the mother is Rh-negative and usually accompanies the laboratory report.

6.5.4 ***Second Blood Sample During Pregnancy***

A second, and sometimes a third, test are made between the 28th and 34th weeks on Rh-negative women and also on Rh-positive women if there is a history of previous transfusions or a history suggestive of haemolytic disease of the newborn.

The presence of antibodies in a mother's serum or a history of a previously affected infant is an absolute indication for confinement in a maternity unit with a pathology laboratory and a special baby-care unit where special investigation and exchange transfusion can, if necessary, be done and where blood of certain special groups is available should the mother need a transfusion.

*Note:* The arrangements for blood grouping tests in connection with pregnancy and documentation differ in detail from region to region. The practitioner should ascertain the local practice from the pathologist or from the Director of the Regional Transfusion Centre.

6.5.5. ***Indications for Examination of Cord Blood***

Although the selection of patients for hospital confinement should ensure that mothers likely to give birth to affected infants are delivered in hospital, some 'missed' cases may be delivered at home. In the presence of any of the following indications at the time of delivery a

sample of cord blood should be collected and sent immediately to the laboratory accompanied by a venous blood sample from the mother :

(a) If the infant appears oedematous, or jaundiced or anaemic or has bruises or if the placenta is bulky and oedematous.

(b) If the first inch or so of the cord at the umbilicus shows greenish-yellow discolouration.

(c) In all cases where the mother is Rh-negative or her blood has not been examined ante-natally.

(d) When examination of the mother's blood during pregnancy has indicated that the child may be born suffering from haemolytic disease.

6.5.6 ***Collection of Cord Blood Sample***

After all necessary attention has been given to the infant, the cord should be clamped. With a dry needle and syringe, the umbilical vein is entered between the clamp and placenta. Ten ml of blood are withdrawn and the needle is removed. Not less than 1 ml for measurement of haemoglobin is gently run into a tube containing Potassium EDTA (Code Pink) and well mixed and the remainder, (for blood grouping and syphilis testing), into a dry sterile tube/tubes (Code White). If a syringe is not available the samples may be collected from the cut end of the cord, taking care to avoid contamination with Wharton's jelly.

6.5.7 ***Husband's Blood and Materials Sensitization***

A sample of the husband's blood (10ml in a dry sterile tube, Code White) need only be taken if the mother herself is anxious to know the prognosis concerning the occurrence of the disease in future pregnancies, or if requested by the pathologist.

## 6.6 Blood Transfusion by the Flying Squad

Blood transfusions are given in hospital save in exceptional circumstances, usually obstetrical emergencies. In the latter circumstances a flying squad, if available, should be called upon to give any treatment by blood transfusion needed before the patient is moved to hospital and full details (from the antenatal record) of the blood group and of any antibodies known to be present should be given when the flying squad is called. A pretransfusion sample of blood (10ml in a dry sterile tube without anticoagulant (Code White) and labelled with the patient's full name, age, address and date) should be collected for subsequent compatibility tests.

6.6.1 ***Intravenous Dextran and Specimen Collection***

In the period before the flying squad arrives, dextran solution or reconstituted dried plasma may be administered. These may be given to recipients of any blood group. If dextran solution is to be given a blood sample for compatibility testing should first be collected, as the presence of dextran may produce pseudo-agglutination. This

will interfere with the subsequent crossmatching tests which would be needed if a blood transfusion were to be necessary later. The flying squad or the hospital laboratory should be informed if dextran has been given.

If transfusion of blood is considered essential before the patient is moved to hospital and a flying squad is not available, the pathologist or regional transfusion director should be consulted.

## 6.7 Dried Plasma

The arrangements for providing dried plasma, distilled water for its reconstitution and giving sets differ from region to region : the pathologist or the Director of the Regional Transfusion Centre will give details of these.

## 7.0 Morbid Anatomy and Histopathology

### 7.1 Transmission of Histopathological Specimens

Small specimens for histological examination should be placed in a wide-mouthed container of suitable size, with at least ten times their volume of formol saline. Larger specimens may be sent in polythene bags containing some formol saline. It is desirable to incise large specimens to allow of fixation.

On no account should tissues be allowed to dry. They should be immersed in fixative immediately after excision. Alcohol or other substances should not be used as fixatives, unless specifically requested by the pathologist. If bacteriology and histology are required on the same specimen it should be divided and portions placed in a plain sterile container in addition to the material put in formol saline.

Most departments supply containers of various sizes filled with formol saline.

## 7.2 Request Forms and Histopathology

As in other sections of laboratory work it is most important for each specimen to be accompanied by a form fully completed ; the form indicates the minimum amount of information required. With gynaecological specimens it is essential to know the menstrual history, the time in the cycle when the specimen was obtained and whether the patient has recently been receiving an oral contraceptive or hormone treatment. Accurate information is also necessary to enable the pathologist to carry out any special examinations he may consider helpful. Great care should be taken with the identification of the specimen, and the container should be labelled with the full name and age.

## 7.3 Post-mortem Examinations

The Department of Health and Social Security has agreed that, where a general practitioner requests a post-mortem examination in the case of a domiciliary death, Hospital Management Committees may pay transport charges for a corpse to be sent to hospital for such examination provided that the hospital pathologist has agreed that an autopsy is necessary.

## 8.0 Cytodiagnosis

### 8.1 Cervical and Vaginal Cytology

Ideally the practitioner should arrange to be given a demonstration by a gynaecologist, of the collection of material from the cervix and vagina, and the preparation and fixation of the smears. The following notes and illustrations are given in the hope that they may be of assistance where this is not possible.

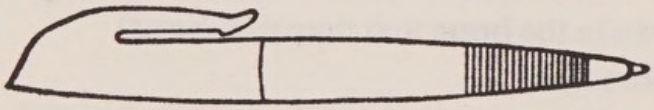
The best time for taking a smear is mid-cycle. Try to avoid the menses. Smears should be taken before a manual vaginal examination is carried out.

Before use the ground-glass end of the slide should be labelled *'lead' pencil* with the patient's name. If it is intended to take vaginal material as well as cervical, mark two slides V and C respectively.

## Technique for Ayre's cervical smear

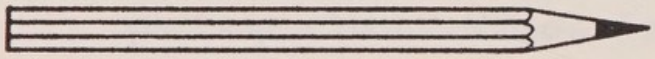
Prepare carbowax fixative

Fill in request form with *ball point pen*



***Request form***

Patient's name in *lead pencil*
on ground glass end of slide



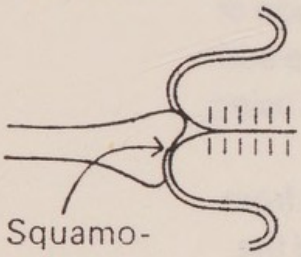
***Slide***

name

Lubricate outer surface of speculum
*very sparingly* with normal saline :
water permissible if saline not available

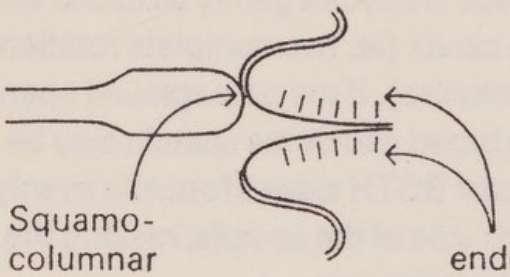
## *Taking the specimen*

*Nulliparous cervix*



Squamo-columnar junction

*Parous or scarred cervix*

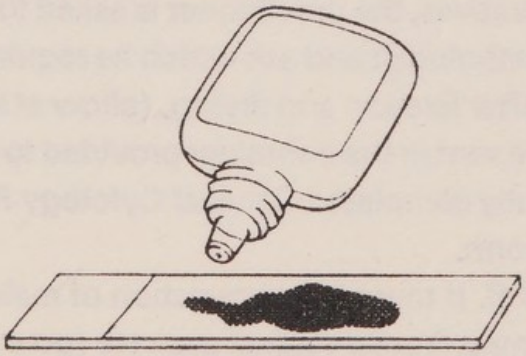


Squamo-columnar junction

endocervical glands

## *Making and fixing the smear*

Wipe off material by gentle even sweeps. Do not go over the same area twice.



Apply fixative immediately while smear is wet. Leave to dry before placing in plastic postal box.

8.1.1 ***Cervical Scrape Technique***

8.1.2 Using a speculum dry, or moistened with warm tap water, (no lubricant) expose the cervix.

8.1.3 In a normal cervix using the bilobed end of the special Ayre's spatula, scrape firmly but gently all round the squamo-columnar junction of the cervix (ie. two complete rotations—one clockwise and one anti-clockwise). If the os is splayed open or scarred a wider sweep using the broad end of the spatula may be needed.

8.1.4 Smear BOTH sides of spatula evenly on slide, with one stroke from each side of the spatula, making the smear on the same side of the slide as the name.

8.1.5 ***Fix immediately.*** As there are differing preferences for fixatives, the practitioner is asked to communicate with his consultant pathologist and ask which he requires.

8.1.6 After fixation and drying, (allow at least 30 minutes) the slides should be sent in the container provided to the laboratory together with a fully completed Cervical Cytology Request Report Form.

8.1.7 *N.B.* If there is any question of malignancy in the body of the uterus, a smear from a vaginal aspirate (posterior fornix) is required. A smear from the lateral vaginal wall is required for information regarding hormone studies on the menstrual cycle.
These slides should be labelled appropriately.

## 8.2 Ascitic and Pleural Fluids Cytology

Fluids for cytological examination should be taken into a tube or bottle containing 1 ml of 3.8 per cent sodium citrate per 25ml of fluid. This is to prevent clotting and to allow the cells to be spun down from the fluid. The specimen should reach the laboratory as soon as possible on the same day. Part of the fluid may be allowed to clot and blocks then prepared for histological examination.

## 8.3 Sputum Cytology

An early morning specimen obtained by a deep cough before drinking, eating or teeth cleaning is required. The patient must be instructed to provide sputum and not saliva.
The sputum is placed in a dry container, securely capped, which must reach the laboratory by mid-day.

## 8.4 Urine Cytology

If the specimen can be examined within 6 hours, collect 2oz of early morning urine and take to the laboratory. If there is likely to be delay in transmission, the early morning specimens should be passed into a 6oz container in which there are 2oz of methylated spirit.

## 8.5 Stomach and Colon Cytology

Washings from these organs are examined at special centres only ; the pathologist will be pleased to advise.

## 9.0 Procedures Recommended for use by General Practitioners

The tests described here are equivalent to the ward side-room tests used in hospital, and can be performed by general practitioners themselves rather than sending the specimens to hospital laboratories. Some practitioners may wish to increase their range of tests—for example, by doing simple haematology or by screening female urine for bacteriuria—but this requires equipment and skills which not all practitioners will find it desirable to acquire. Such tests are not described here.

### 9.1 'Stick' or 'Tape' Tests for Urine

The best known are the '... stix' range produced by the Ames Co but Eli Lilly produce 'Testape' for glucose, and the Boehringer Co, a series 'BM Test': the last is not at present being distributed in Britain. The manufacturers instruction brochures should be consulted for detailed instruction.

The '... stix' range include :

9.1.1 ***'Albustix'*** A semi-quantitative test for protein : it is especially sensitive for albumin, but may give underestimates or negative results with globulin or Bence Jones protein, and overestimates or false positives with very alkaline urine, and with Cetavlon and other quaternary ammonium (cationic) detergent/antiseptics which are sometimes used to clean urinals or urine glasses.

9.1.2 ***'Clinistix' (also 'Testape')*** A test for glucose : a highly specific glucose oxidase test, it is inhibited by high concentrations of ascorbic acid. *It is not quantitative, and does not detect other reducing substances* (eg. galactose). 'Clinitest' (or Benedict's reagent) should therefore *always* be used for initial urine testing in infants and for approximate quantitation if required for control of diabetic patients. (Section 9.2.4).
Oxidising cleansing agents may give false positive reactions.

9.1.3 ***'Ketostix'*** A specific test for ketone bodies. It detects 5-10mg/100ml and above of acetoacetic acid in urine.

9.1.4 ***'Phenistix'*** A test for phenylpyruvic acid. Reliable for detecting the acid at concentrations above 10mg/100ml *but not for the diagnosis of phenylketonuria.* The Guthrie test on heel-prick blood on filter paper is now the recommended screening procedure but some pathologists prefer capillary tube blood and other methods.

9.1.5 ***'Hemastix'*** A test for blood, free haemoglobin and myoglobin in urine.

9.1.6 ***'Ictostix'*** A specific test for bilirubin in urine which is a little less sensitive than Ictotest tablets (Section 9.2.6) but is semi-quantitative.

9.1.7 ***'Urobilistix'*** A semi-quantitative test for urobilinogen in urine, which does not react with urobilin in concentrations that occur in urine.

9.1.8 ***'Bili-Labstix'*** A stick test which tests simultaneously for pH, protein (see comments on Albustix Section 9.1.1) glucose (not reducing substances), blood, bilirubin and ketone bodies in urine. Other combinations are available, and with all of them care must be taken to avoid confusion between the different reagent zones : for this reason many find the single tests more satisfactory.

## 9.2 Other Methods of Testing Urine

Most of these are relatively slow and messy, but they are sometimes useful to confirm unexpected results, especially if it is not practicable to refer the sample to a hospital laboratory.

Before using any of these except the urine blood tests, the urine should be clear, or be cleared by filtration : a No. 1 Whatman filter paper is satisfactory. Exceptionally a deposit may be allowed to settle and the clear supernatant used.

9.2.1 ***Specific Gravity of Urine*** The 'Urinary Densimeter' is a clean and convenient instrument. It is a plastic tube with a nozzle, which is fitted with an aspirating bulb and contains coloured plastic beads of sp gr 1.005, 1.010, 1.015, 1.020 and 1.025. There are also on the market refractive index instruments which are very convenient, but expensive. The floating Urinometer is the traditional instrument. The urinometer must not touch the sides of the container and should have its zero checked occasionally with distilled water. If the specimen is too small to float the urinometer it can be diluted with an equal volume of water, and the last two figures of the sp gr of the mixture doubled. The presence of glucose increases the sp gr by 0.004 for each 1%, and of protein by 0.003 for each 1%.

9.2.2 ***pH of Urine*** pH sensitive indicator paper (BDH : Johnson) is distributed in plastic dispensers. A piece is dipped in the urine and after 30 seconds the colour compared with the chart on the dispenser. The 'Full range' paper covers pH 1-14 but is rather insensitive at pH 3-7. More sensitive narrow-range indicators covering pH 4-6 and pH 6-8 are available if needed. Litmus paper is not suitable for testing.

9.2.3 ***Protein in Urine***

(a) To clear urine add 1/10 volume 25% salicylsulphonic acid and mix. After 1 minute compare with a blank against a dark background, looking at the samples from a direction at right angles to

the path of the illuminating light. Limit of detection about 10mg/100ml. False positive results may be obtained with tolbutamide and some materials used for excretion pyelograms.

(b) Make 10ml urine slightly acid (pH 3-4) by adding 33% acetic acid drop by drop. Mix and divide into two portions. Boil one in a flame or heat in a boiling waterbath for 5 minutes, then cool. Compare the two tubes as in method (a). Limit of detection about 10mg/100ml.

9.2.4 ***Reducing Substance in Urine***

(a) 'Clinitest' (Ames Co). A tablet equivalent of Benedict's test, limit of detection 0.2%. Place 5 drops urine and 10 drops water in a tube, drop in one tablet. Compare with colour chart soon after boiling has ceased.

(b) Benedict's test. To 5ml Benedict's qualitative solution in a test tube add 0.5ml urine, mix and place in boiling waterbath for 5 minutes. Stand for 2 minutes. A greenish-yellow to brick-red precipitate indicates reducing substance, usually glucose—which can then be confirmed by a specific test. Complete decolorization of supernatant liquid with red precipitate = 2% or more : limit of detection about 0.1%.

9.2.5 ***Ketone Bodies in Urine***

(a) Gerhardt's test. To 5ml urine add drop by drop about 1 ml reagent

(10% ferric chloride in 2N HCl). A plum coloured solution indicates acetoacetic acid. Salicylates and p-amino salicylic acid give violet colours often difficult to distinguish from acetoacetic acid. 'Acetest' or 'Ketostix' can be used to differentiate because they are specific for Ketones, or a further sample of urine can be boiled for 10 minutes in an open dish when acetoacetic acid will decompose.

Phenylpyruvic acid, and some other phenolic compounds, give green colours and some drugs give other colours, eg. chlorpromazine (mauve).

In the absence of drugs a positive test is pathognomonic of severe ketosis.

(b) Acetest (Ames Co). A tablet test similar to but less sensitive than Rothera's test. Use according to maker's instructions. 'Ketostix' (Section 9.1.3 Ames Co) is based on the same principle.

9.2.6 ***Bile Pigments in Urine***

(a) Bilirubin. Ictotest (Ames Co). A specific diazo tablet test. Use according to the maker's instructions. The limit of detection is about 0.1 mg/100ml.

(b) Urobilinogen (or Porphobilinogen). Watson and Schwartz modification of Ehrlich's test. To 1 ml fresh urine add 1 ml reagent. (0.7g p-dimethylaminobenzaldehyde in a mixture of 150ml

concentrated hydrochloric acid and 100ml water). Allow to stand for 3-5 minutes. Add 2ml of saturated sodium acetate solution and mix. A red colour indicates either excess urobilinogen or porphobilinogen—(a trace of urobilinogen is normal). This colour reaction is rather unspecific and weakly positive results are obtained after the ingestion of many drugs and foodstuffs. 'Urobilistix' (Section 9.1.7 Ames Co) is based on the same reaction.

9.2.7 ***Calcium in Urine*** Sulkowich test. Mix equal volumes of urine and reagent and stand for 2-3 minutes.
No precipitate indicates no calcium. Fine white cloud indicates normal. Heavy precipitate indicates excess.
*Reagent:* 2.5g oxalic acid, 2.5g ammonium oxalate and 5ml glacial acetic acid made up to 150ml with water.

9.2.8 ***Chloride in Urine*** To 5ml urine add 1ml 10% nitric acid and 1-2ml silver nitrate (concentration between 2 and 5%). A white curdy precipitate is given by normal concentrations of chloride. No precipitate, or a light cloud, suggests salt depletion. This test is useful in testing for chloride deficiency due to sweating in heat exhaustion.

9.2.9 ***Erythrocytes in Urine*** Microscopy on the centrifuged deposit is more sensitive to erythrocytes than are some chemical tests, but it does not detect free haemoglobin or myoglobin.

9.3 **Phenylpyruvic Acid in Urine** There are no completely reliable screening tests, but those outlined here are the best if a blood sample cannot be obtained for screening. (Blood phenylalanine assays are necessary for the definitive diagnosis of phenylketonuria and the Guthrie bacterial inhibition assay on heel-prick blood collected on special filter-paper is to be preferred to either of the following tests and is generally available).

(a) Ferric chloride. Add a few drops of reagent to 5ml urine in a test tube. A green colour, fading after 2 minutes, suggests phenylpyruvic acid. The urine should be as fresh as possible and not alkaline as the keto-acids decompose readily in alkaline solution.

*Reagent:* 10% forric chloride in 0.25 N hydrochloric acid.

(b) Phenistix give colours similar to the ferric chloride test.

9.4 **'Stick' Tests for Blood** The following tests in the '.....stix' range are also produced by Ames Co for use with whole blood.

9.4.1 ***'Dextrostix'*** A semi-quantitative stick test for blood glucose levels. Use in accordance with makers' instructions.

9.4.2 ***'Azostix'*** A semi-quantitative test for blood urea levels. Use in accordance with makers' instructions.

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