

South London Specialist Virology Centre

VIROLOGY

Laboratory User's Handbook Feb 2009 version



www.kingspath.co.uk



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www.clinical-virology.org

Health Protection Agency London – Regional Laboratory
South London Specialist Virology Centre
in conjunction with
Department of Medical Microbiology

Prepared by Dr M Sudhanva, Consultant Virologist

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1. General information

The **Health Protection Agency London** is a HPA Regional Laboratory based at Kings College Hospital NHS Foundation Trust. It provides an extensive clinical microbiology service, including infection control services and specialist advice in microbiology, virology, parasitology and mycology to hospitals and General Practitioners. South London Specialist Virology Centre is the virology part of HPA Regional Laboratory and UK clinical virology network

Epidemiological data are provided for the Communicable Disease Surveillance Centre in Colindale. Outbreaks of infectious disease are investigated in conjunction with the Consultants in Communicable Disease Control.

The Health Protection Agency London belongs to National Quality Assurance Schemes and it is accredited by the Clinical Pathology Accreditation (CPA) scheme.

1.1 Where to find the virology

Postal address

South London Specialist Virology Centre Health Protection Agency London Regional Laboratory King's College Hospital NHS Foundation Trust Cheyne Wing, 2nd Floor Denmark Hill London SE5 9RS	DX address: South London (PHL) Kings DX 6570200 Peckham 90SE
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1.2 Population served

King's College Hospital NHS Foundation Trust provides a wide range of services to the population of South East London. During 2007, approximately 255,000 specimens were received in the Health Protection Agency London. The Trust serves Lambeth, Southwark and Lewisham with a population of 700,000. Public Health microbiology is provided for a resident population of approximately 1.5 million.

King's College Hospital is a teaching hospital and centre of excellence with 950 in-patient beds, several specialist units of international repute, and units offering regional or supraregional services. The Trust has a budget of £471 million, employs 6,000 staff and cares for 415,000 patients annually – (85,000 in A&E). King's College Hospital works closely with other healthcare organisations, such as local Primary Care Trusts. We also have strong academic links with King's College London together with our Academic Health Sciences partners, Guy's and St Thomas' and the South London and Maudsley NHS Foundation Trusts.

The regional and supraregional services include:

- Haemato-oncology (including UK's largest bone marrow transplantation unit)
- Institute for Liver Studies (providing 30% of UK liver therapy, including transplantation and liver failure) for both adult and paediatric hepatology
- Variety Club Children's Hospital
- Regional Neurosciences and Neurosurgical services
- Renal unit (offering dialysis for HBV, HCV and HIV positive individuals)
- Harris Birthright Centre for Foetal Medicine
- Adult Intensive Care Unit
- General neonatal and paediatric intensive care
- Solid tumour oncology / cancer services (including skin, hepatobiliary, head and neck)
- Cardiac surgery (regional)
- Paediatric and adult Accident and Emergency departments
- Obstetrics and gynaecology; assisted conception
- Genito-urinary medicine (Caldecot Centre)
- Reproductive and Sexual Health

1.3 Primary care

The laboratory serves more than 100 fund-holding general practitioners. The response of King's College Hospital to the evolving Primary Care Groups and Trusts has imposed further demands on the clinical virology service. The King's local Primary Referral Guide reflects general practitioners' expectations of direct access for prompt clinical advice, including virological advice. Specialist virology services and clinical advice are offered by virology to the London Boroughs of Lambeth, Southwark, Lewisham, Bromley, Bexley and Greenwich. The local authorities serviced by the laboratory are the London Boroughs of Bromley, Southwark, Bexley and Greenwich.

1.4 South London Specialist Virology Centre

Virology includes two Consultant Virologists, two Specialist Registrars, a Principal Clinical Scientist, three more Clinical Scientists, a Head of Operations, Laboratory Chief, a BMS-3++, 4 BMS-2, 10 BMS-1 and 6 SATOs, supported by quality manager and administrative & clerical staff. The team manages a workload of over 100,000 samples of increasing complexity and requiring sophisticated laboratory processing and interpretation. The routine diagnostic work includes immunofluorescence, general serology, hepatitis and retrovirus serology, and molecular based tests which include both qualitative and quantitative assays as well as antiretroviral resistance testing using automated sequence analysis. Most molecular in-house PCRs are carried out using Rotor-Gene and LightCycler technology. General microbiology serological tests which are sent to the combined department are carried out in virology. In addition, all *Chlamydia trachomatis* and *N gonorrhoea* tests are carried out by APTIMA (TMA) technology on genital swab / urine samples. Virology receives specimens from a number of external microbiology laboratories for investigation.

1.5 Research

There are collaborations between clinical research groups and academic partners at Guy's, King's, and St Thomas' School of Medicine, the Institute of Psychiatry, The School of Nursing and Midwifery, King's College London and King's Division of Biomedical Sciences.

Research interests in the laboratory include:

The Principal Clinical Scientist, Dr Melvyn Smith along with grade B Scientist Dr Andrea Szendroi, manage the molecular diagnostic section for virology (along with the BMS staff). Dr M Smith supervises projects carried out by biomedical scientists and other clinical scientists in virology together with specialist registrars in both departments. They are involved in a variety of projects, many of which have resulted in technology transfer to the routine diagnostic service. Work carried out in the departments has been published in numerous peer-reviewed journals and presented at local, national and international meetings.

There are collaborations between clinical research groups and academic partners at Guy's, King's, and St Thomas' School of Medicine, the Institute of Psychiatry, The School of Nursing and Midwifery, King's College London and King's Division of Biomedical Sciences.

The development of real-time polymerase chain reaction assays, using the Corbett Rotor-Gene, Roche LightCycler and Bio-Rad iCycler, which is centred on the population of patients attending the tertiary referral services at King's. In particular, assays have been developed for quantitative CMV DNA and adenovirus DNA detection and quantification, EBV DNA quantification and the detection and typing of HSV types 1 and 2 DNA. Multiplex assays are used to detect a range of respiratory pathogens, including influenza A and B viruses, RSV subtype A and B, human metapneumovirus, human rhinovirus and parainfluenza 1, 2 & 3 viruses. New assays include norovirus detection and hepatitis B virus detection and sequencing, in order to determine any HBV drug resistance mutations present. HIV-1 subtype analysis of the local HIV infected cohort using in-house sequencing methods and comparisons of HIV-1 RNA assays have also been carried out. As part of a collaborative PhD project with the Institute of Liver Studies, we are also developing DNA arrays and flow cytometry methods to investigate the natural history of EBV infection, particularly related to paediatric liver transplant recipients.

Further collaborative projects with bacteriology have led to the development of assays to detect nucleic acid from *Pneumocystis jirovecii*, *Haemophilus influenzae*, *Aspergillus fumigatus*, MRSA and erythromycin resistance genes in beta-haemolytic streptococci. We are also one of only 12 laboratories in UK accredited to carry out avian influenza A virus H5N1 testing.

Surveillance activity in virology.

The laboratory is also involved in a number of anonymous serological surveys. The laboratory is also the lead laboratory for the London region for the Enhanced Flu Surveillance program which involves respiratory virus surveillance of patients attending GP surgeries with respiratory illnesses. At the moment 4 GP surgeries around Kings and 4 GP surgeries in the Bromley PCT area send samples for this surveillance. The laboratory also assesses faecal samples from suspected norovirus outbreaks in the South Thames region.

1.6 Key personnel and contact details

Virology direct lines: Results Phone: 020 3299 6155 Fax: 020 3299 6477
Reception: 020 3299 6214
Medical advice: 020 3299 6298

Microbiology Phone: 020 3299 3213 / 3565 Fax: 020 3299 3404

Virology only - 020 3299 9000 plus extension		
Designation	Name	Telephone extension
Head of Virology Consultant Virologist	Dr Mark Zuckerman	6296 / 6970
Consultant Virologist	Dr M Sudhanva	6296 / 6971
BMS 3 (Virology)	Mr Alan Spratt	6159

Microbiology - 020 3299 9000 plus extension		
Designation	Name	Telephone extension
Director Consultant Microbiologist	Dr Jim Wade	3033
Chairman Senior Lecturer	Dr John Philpott-Howard	3256
Consultant Microbiologist	Dr Amanda Fife	3095
Consultant Microbiologist and Infection Control doctor	Dr Ian Eltringham	3766
Consultant Microbiologist	Dr Dakshika Jeyaratnam	2569

Microbiology and Virology combined - 020 3299 9000		
Head of Operations	Mr Ross McEwan	6259
Principal Clinical Scientist	Dr Melvyn Smith	6155
Senior Infection Control Nurse	Mr Bill Houston	4374
Laboratory Administrator	Mrs Linda Akkad	6260
Quality Manager	Mr Craig Smith	6140

KingsPath Blood Sciences Laboratory - 020 3299 9000		
Laboratory Manager	Mr Frank Oliyide	1659
BMS 2	Mr Ralph Henderson	2217

1.7 Normal laboratory opening times

Routine specimens are accepted at the virology laboratory from:
Monday to Friday 9 AM to 5 PM

2 Use of the Laboratory

2.1 Test requests

2.1.1 Requesting procedure (routine, urgent and out of hours)

Routine requests can be made either by King's EPR system or any virology / microbiology request form. During normal laboratory hours please telephone urgent requests (020 3299 xxxx either 6214, 6155 or speak to the medical staff on 6298) to ensure priority processing. See section 3 for use of on-call services.

2.1.2 Completing the request form

EPR requests:

Most King's samples requests can be made via EPR. Please free text in the clinical details field if you can not see an intended test on EPR request.

Types of non-EPR request forms:

Blue virology form	⇒	⇒	⇒	⇒GP / Internal use
Yellow request form	⇒	⇒	⇒	⇒GUM / GP / Antenatal
Multipart single pathology request	⇒			⇒GP use
White virology form	⇒	⇒	⇒	⇒HIV plasma viral load testing (EPR ordering available)
Specialised request form	⇒	⇒		⇒HIV antiretroviral resistance testing (EPR ordering available)

The form should be completed legibly. Please use labels whenever possible.

A request form must accompany all specimens sent to the laboratory. It should clearly state the following information:

- Patient name and address
- Unit number/NHS number
- Date of birth (age if DOB not known)
- Sex
- Ward
- GP name and number
- Address for report
- Bleep number or mobile number, in order to phone results both before 5 PM and after 5 PM results
- Type of specimen
- Date and time specimen taken
- Tests required
- All relevant clinical details including any antimicrobial treatment (recent, current and intended)
- History of foreign travel including return dates
- Viral Haemorrhagic Fever Risk status if applicable – **MUST BE DISCUSSED WITH MEDICAL STAFF BEFORE SENDING SAMPLE**
- Date of onset and duration of illness, particularly for serology
- Specify anatomical site from which vesicle swab / fluid specimens were taken
- Useful epidemiological information, e.g.:
 - Children and suspected influenza - give the name of the school
 - Adults and suspected norovirus - give the place and type of work, (e.g., catering, cruise liner)
 - All patients and suspected viral haemorrhagic fever – travel destination, date of return, signs and symptoms, malaria smear

If uncertain about the exact test and terminology, please give a detailed clinical history as this can help the Virology medical staff to decide the most appropriate investigations.

2.1.3 Specimen labelling

- The specimen must be labelled with the same patient details as that on the request form
- Please note that unlabelled specimens do not guarantee authenticity of the sample and these cannot be processed and these may be discarded.

2.1.4 Specimen collection

The best results are obtained when an appropriate, well-taken specimen is in the proper container, is delivered to the laboratory promptly and relevant clinical information provided on the request form. Please contact the laboratory if there is any doubt about the best specimen to take or you have questions about any test.

General guidelines on specimen collection include:

- Send specimens in sterile containers
- Collect specimens from the actual site of suspected infection. Please do not send just blood samples for 'viral serology' instead of vesicular fluid or throat swab or CSF, as the case may be.
- Take specimens that are representative of the disease process. For example, respiratory specimens are more appropriate than blood for serology in cases of acute respiratory infection.
- An adequate quantity of material should be obtained for complete examination especially vesicle fluid, CSF and NPA
- Take care to avoid contamination of the specimen by micro organisms normally found on the skin and mucus membranes. Sterile equipment and aseptic technique must be used for collecting specimens, particularly for those from normally sterile sites
- All swabs or material from swabs should be immersed in virus transport medium (VTM) and transported promptly to the laboratory. Viruses including (viral nucleic acid) may not survive prolonged storage at room temperature or may be overgrown by bacteria or fungi.
- In the absence of readily available VTM (laboratory issued or commercial), please immerse the swab tips in 1 ml of sterile saline in an universal container

2.2. Transport to the laboratory

2.2.1 Receipt of specimens

During normal working hours, all routine King's specimens should be taken to central specimen reception at King's College Hospital. For urgent testing bring the specimen DIRECTLY to the laboratory reception, which is on the 2nd floor of Cheyne Wing (opposite Liver ITU). Do not use Central Specimen Reception for urgent testing, but contact laboratory or on-call BMS staff for directions before arranging urgent transport to laboratory reception. See section 2.2.3 for samples referred from external institutions and laboratories.

2.2.2 High risk specimens and safety

Pathogens are classified in hazard groups 1 to 4, with hazard group 1 being non-pathogenic to humans and hazard group 4 the most dangerous pathogens to humans.

All blood samples from suspected HIV and hepatitis patients are handled safely in the laboratory and consequently **we do not require "DANGER OF INFECTION" labels**. It is the responsibility of the sender to pack samples safe enough for transportation according to regulations.

We assume all respiratory samples may potentially contain a hazard group 3 pathogen and treat samples accordingly. However, samples from patients with suspected viral haemorrhagic fevers, with a history of having returned within 21 days from Africa, Asia and South America are considered high risk. Contact virology medical staff before taking ANY sample. Special transport arrangements of these samples will be made in conjunction with HPA, Porton Down.

Great care must be taken in obtaining specimens. Equipment such as needles and blades must be immediately disposed of safely in locally approved "sharps" bins and NOT SENT TO LABORATORIES. Specimens should be transported to the laboratory without delay.

2.2.3 Courier and postal deliveries

When sending samples from an external institution or laboratory, it is the responsibility of the sender to ensure that the samples are packed in accordance with the current postal regulations, contain appropriate paper work and are labelled correctly. Courier / taxi / suitable transport should be arranged by sending institution or laboratory. You may have to contact the on-call BMS staff for out-of-hours' testing to indicate approximate time of arrival of sample at virology. Our experience shows that considerable amount of time is wasted by our on-call BMS staff just waiting for a sample to arrive because of lack of communication from test requesting person.

2.3 Results and reports

2.3.1. Printed results

Electronic reports are exported to EPR within 100 seconds of authorising.

Non-EPR reports are printed and dispatched every working day – Monday to Friday. The speed of reporting depends on the frequency of testing and the urgency of the request.

Copies of printed reports can be obtained by phoning extension 6155. Reports are never faxed.

2.3.2. Telephoned results

Results of urgent requests and rapid requests that may aid the immediate patient management will be telephoned.

Examples include:

- Respiratory virus detection by direct immunofluorescence / PCR in a respiratory sample.
- CSF nucleic acid test positive result
- Any acute infection diagnosis
- Blood borne virus infection such as new HIV, acute HBV, acute HCV and HTLV

2.4 Visitors

Visitors should introduce themselves at the laboratory reception (2nd floor, Cheyne Wing, opposite Liver ITU). The person they wish to see will come to meet them. It is best to make appointments in advance to ensure the right person is available.

3 Out of hours' service

3.1 Out of hours' examinations provided in virology

An on-call service is provided by the Virology from 5pm to 9am Monday to Friday, all day Saturday, Sunday and bank holidays. For urgent specimens, contact the doctor or BMS on-call through the KCH switchboard (020 3299 9000). Transplant coordinators should contact the King's College Hospital switchboard (020 3299 9000) and ask for the on call biomedical scientist.

Urgent / out of hours requests in virology

Assays / samples	Specimen type	Turn around time
HIV antibody	10 ml clotted blood	2 hours
Hepatitis A virus IgM	10 ml clotted blood	2 hours
Hepatitis B surface antigen	10 ml clotted blood	2 hours
Hepatitis B core total antibody	10 ml clotted blood	2 hours
Hepatitis B core IgM antibody	10 ml clotted blood	2 hours
Hepatitis B surface antibody	10 ml clotted blood	2 hours
Hepatitis C virus antibody	10 ml clotted blood	2 hours
HTLV I / II	10 ml clotted blood	3 hours
CMV IgG antibody	10 ml clotted blood	2 hours
Measles virus IgG	10 ml clotted blood	4 hours
Respiratory virus detection by DIF	NPA / BAL	2 hours
Avian influenza A H5N1 PCR	Combined throat and nasal swab	4 hours

Other tests can be carried out after discussion with one of the medical virologists.

3. 2 Medical advice regarding the diagnosis and treatment of infection

During weekdays from 9 am to 5 pm medical advice on interpretation of virology results, antiviral management, blood borne virus exposure incidents and post exposure prophylaxis or any other relevant clinical circumstance can be sought from the virology specialist registrars or consultants on extension 6298. Please **DO NOT** call 6298 to obtain **RESULTS** (for results call 6155).

3.3 Out of hours' advice

An on-call service is provided by the Virology from 5pm to 9am Monday to Friday, all day Saturday, Sunday and bank holidays. To arrange for samples to be sent to please contact the King's College Hospital switchboard (020 3299 9000) and ask for the on call biomedical scientist. The on-call biomedical scientists will not look up results out of hours unless discussed and agreed with the medical virology staff.

Out of hours a Specialist Registrar and/or Consultant Virologist can be contacted via KCH switchboard to discuss clinical, diagnostic and therapeutic problems with doctors.

Consultants: Dr Mark Zuckerman and Dr M Sudhanva

SpR: Dr Samuel Moses

ST1: Dr Stephen Winchester

During working hours the Registrar on clinical rota is available on 078330 46880

During the day infection control advice can be obtained from the Micro Web or from the Infection Control Nurses on extension 3896.

Appropriate specimens for urgent examination on-call include:

- Urgent nasopharyngeal aspirate / BAL investigation for respiratory viruses
- Urgent duplicate combined throat and nasal swabs for avian influenza A H5N1 virus
- Screening of organ transplant donors and renal dialysis patients
- Other tests are available after consultation with medical virology staff

4. Sections within the virology

Within the Virology Department there are two diagnostic sections viz. Virus Identification Section and Virus serology.

4.1 Virus Identification Section

This section comprises of virus isolation and molecular diagnostics.

Virus isolation is now rudimentary and we no longer provide routine virus isolation service. An on-call direct immunofluorescence (DIF) is still provided, however it is not a routine service. Samples for electron microscopy (EM) are referred to another laboratory if needed.

Molecular diagnostics use real time PCR (Rotor-Gene, LightCycler and iCycler) and block based techniques for the detection of viral nucleic acid in clinical samples. Nucleic acid tests performed routinely are for the detection and quantification of HIV-1 RNA, CMV DNA and EBV DNA, qualitative detection of avian influenza virus A H5N1, HSV DNA, VZV DNA, adenovirus DNA, enterovirus RNA, HCV RNA (quantitative) and *C. trachomatis* & *N gonorrhoea* nucleic acid. Norovirus RNA testing of stool samples by PCR is the latest addition.

Multiplex PCRs are available for the following viruses as routine testing of all respiratory samples along with stand alone adenovirus DNA testing:-

multiplex 1 : influenza A virus, influenza B virus and human metapneumovirus,

multiplex 2 : RSV subgroup A and B, rhinovirus

multiplex 3 : parainfluenza viruses 1, 2 and 3.

Antiretroviral resistance testing by nucleic acid sequencing and HBV DNA sequencing for resistance testing are also provided.

4.2 Virus serology

The virus serology section detects antibodies to viruses and viral antigens in serum using manual and automated assays. In addition, few bacterial antibodies are also detected in this section. Automated machines include TECAN, AxSym, VIDAS and ETIMAX.

Blood Sciences Laboratory, KingsPath <http://www.kingspath.co.uk/>

HPA Regional Laboratory is also part of KingsPath, which is the Clinical Diagnostic Pathology Service at King's College Hospital (www.kch.nhs.uk). KingsPath has now come to an end of a major re-

development programme which has seen the investment of £8m in our pathology service. This includes investment in a state-of-the-art Blood Sciences Laboratory. The Blood Sciences Laboratory, completed in October 2006, is one of the largest, integrated, automated laboratories in Europe, ensuring KingsPath remains at the forefront of developments in Pathology. Since Dec 2007, the following tests on blood are now being carried out by Blood Sciences Laboratory; all antibacterial levels, rubella virus IgG and IgM, hepatitis A virus total antibody and IgM, HBs antibody level, hepatitis B core total antibody and hepatitis C virus antibody.

5. List of examinations performed in virology (all turn round times in working days)

5.1 Virus detection and isolation

Specimen type	Tests performed	Schedule	Turn round time
Nasopharyngeal aspirate	Direct detection by DIF	on call only	2 – 4 hours
Broncho-alveolar lavage	Direct detection by DIF (on call only)	on call only	2 – 4 hours
Genital swab	Cell culture (for obtaining and isolate for HSV drug resistance testing)	When necessary	2 - 7 days

Please note the virus isolation is no longer a routinely performed

5.2 Viral nucleic acid tests

Molecular assays on blood

Test	Specimen type	Schedule	Turn round time
CMV DNA - quantitative	EDTA	Daily	2 - 4 days
EBV DNA - quantitative	EDTA	Twice a week	3 - 5 days
VZV DNA	EDTA	Daily	1- 3 days
HSV 1 and 2 DNA	EDTA	Daily	1 - 3 days
Enterovirus RNA	EDTA	Once a week	2 - 7 days
Adenovirus DNA	EDTA	Twice a week	3 - 5 days
HCV RNA	EDTA / clotted	Once a week	5 - 14 days
HIV-1 RNA viral load	EDTA	Daily	5 - 14 days
HIV-1 proviral DNA (paediatric patients)	EDTA	Sent away	14 – 28 days
HIV-1 antiretroviral resistance (email medics for urgent testing)	EDTA	Twice a month	14 – 28 days

Molecular assays on other samples

Test	Specimen type	Schedule	Turn round time
<i>Chlamydia trachomatis</i> and <i>Neisseria gonorrhoea</i>	Endocervical swab or urine or urethral swab	Daily	3 – 5 days
<i>Chlamydia trachomatis</i>	Eye swab	Daily	7 days
CMV DNA	CSF	Daily	2 - 4 days
EBV DNA	CSF	Twice a week	3 - 5 days
VZV DNA	CSF and swab	Daily	1- 3 days
Adenovirus DNA	CSF	Twice a week	3 - 5 days
HSV 1 and 2 DNA	CSF, swab, eye swab	Daily	1 - 3 days
Enterovirus RNA	CSF	Once a week	2 - 7 days
JC virus DNA	CSF	Sent away	14 days
BK virus DNA	Urine and blood	Sent away	14 days
Norovirus	Stool	Daily	1 – 2 days

Molecular assays on other samples contd.

Influenza A virus	Combined throat and nasal swab in VTM, NPA or BAL	Daily	1- 4 days
Influenza B virus			
Avian influenza A virus H5N1			
Parainfluenza 1 virus			
Parainfluenza 2 virus			
Parainfluenza 3 virus			
Respiratory syncytial virus (RSV) subgroup A			
Respiratory syncytial virus (RSV) subgroup B			
Rhinoviruses			
Human metapneumoviruses			
Adenovirus DNA			
HBV DNA antiviral drug resistance	EDTA blood	Weekly	1 – 2 weeks
HSV DNA	Eye / conjunctival / corneal Swab	Daily	2 - 7 days
Adenovirus DNA			
VZV DNA			

5.3 Virus serology including bacterial serology

Test	Primary test performed at	Clotted blood	Schedule	Turn round time (TRT)
Hepatitis A virus IgM antibody	BSL	10 ml	Daily	2 days
Hepatitis A virus IgG antibody	BSL	10 ml	Daily	3 days
Hepatitis B surface antigen**	HPA	10 ml	Daily	2 days
Hepatitis B surface antigen confirmation by neutralisation	HPA	10 ml	Daily	3 days
Hepatitis B core antibody	BSL	10 ml	Daily	2 days
Hepatitis B core IgM	HPA	10 ml	As required	3 days
Hepatitis B e antigen	HPA	10 ml	As required	3 days
Hepatitis B e antibody	HPA	10 ml	As required	3 days
Hepatitis B surface antibody	BSL	10 ml	Daily	2 days
Hepatitis C virus antibody**	BSL	10 ml	Daily	2 days
Hepatitis C virus antibody by RIBA	BSL	10 ml	As required	7 days
HIV antibody**	HPA	10 ml	Daily	2 days
HIV antibody - confirmation	HPA	10 ml	Daily	2 days
HIV-1p24 antigen - confirmation	HPA	10 ml	Daily	2 days
HTLV 1 antibody	HPA	10 ml	Weekly	3 days
HTLV 1 antibody – confirmation (immunoblot)	HPA	10 ml	As required	7 days
CMV IgG antibody	HPA	10 ml	2- 3 times a week	3 days
CMV IgM antibody	HPA	10 ml	2 - 3 times a week	3 days
EBV VCA IgG antibody	HPA	10 ml	2 - 3 times a week	3 days
EBV VCA IgM antibody	HPA	10 ml	2 - 3 times a week	3 days
EBV IgM antibody – confirmation by IF	HPA	10 ml	As required	4 days
HSV IgG antibody	HPA	10 ml	As required	7 days
HSV antibody type specific IgG	HPA - Colindale	10 ml	As required	14 days
Measles IgM antibody	HPA - Colindale	10 ml	As required	1 – 14 days
Measles IgG antibody	HPA	10 ml	As required	1 – 14 days
Mumps IgM antibody	HPA - Colindale	10 ml	As required	1 – 14 days
Mumps IgG antibody	HPA	10 ml	As required	1 – 14 days

Test	Primary test performed at	Clotted blood	Schedule	Turn round time (TRT)
Parvovirus B19 IgM antibody	HPA	10 ml	1 - 2 times a week	4 days
Parvovirus B19 IgG antibody	HPA	10 ml	1 - 2 times a week	4 days
Rubella virus IgM antibody	BSL	10 ml	2- 3 times a week	3 days
Rubella virus IgG antibody	BSL	10 ml	Daily	2 days
Rubella IgG antibody - confirmation	HPA	10 ml	As required	3 days
Varicella zoster virus IgG antibody	HPA	10 ml	2 - 3 times a week	2 days

Respiratory Complement Fixation tests

(We will be offering limited CFT testing service in the very near future)

CFT - Influenza A virus antibody	HPA	10 ml	Once a week	7 – 14 days
CFT - Influenza B virus antibody	HPA	10 ml	Once a week	7 – 14 days
CFT - Adenovirus antibody	HPA	10 ml	Once a week	7 – 14 days
CFT - RSV antibody	HPA	10 ml	Once a week	7 – 14 days
CFT - Q fever antibody	HPA	10 ml	Once a week	7 – 14 days
CFT – Chlamydia genus antibody	HPA	10 ml	Once a week	7 – 14 days
CFT - Mycoplasma antibody	HPA	10 ml	Once a week	7 – 14 days

Bacterial serology – testing service provided by virology, clinical advice by microbiology

Treponemal (syphilis) total antibody**	HPA	10 ml	Daily	2 – 5 days
Treponemal (syphilis) RPR	HPA	10 ml	Daily	2 – 5 days
Syphilis IgM antibody	HPA	10 ml	Daily	2 – 5 days
Toxoplasma IgG antibody	HPA	10 ml	3 - 4 times a week	2 – 14 days
Anti-streptolysin O (ASO)	HPA	10 ml	Once a week	7 days
Aspergillus antigen (Galactomannan)	HPA	10 ml	Weekly	7 - 14days

*One 10ml clotted blood sample is usually sufficient for multiple serology tests

**Patient consent must be obtained by the requesting clinician.

5.4 Serology battery tests

For certain patient groups the following tests will be performed:

Requests	Tests Performed
Acute hepatitis (jaundice, raised / abnormal LFTs)	HAV IgM, HBV surface antigen, HCV antibody – routine. EBV VCA IgM, CMV IgM – are optional.
Miscarriage / TORCH	CMV IgM, CMV IgG, rubella virus IgM, rubella virus IgG, parvovirus B19 IgM, parvovirus B19 IgG
Previous / past hepatitis	HAV IgG, HBV core total antibody, HCV antibody
Viral screen before transplantation	HCV antibody, CMV IgG, EBV VCA IgG, treponemal (syphilis) IgG, Toxoplasma IgG, HTLV 1 and 2 IgG, VZV IgG, HBV surface antigen, HBV core total antibody, HIV 1 and 2 antibody
Pregnancy screening (booking blood)	Treponemal (syphilis) IgG, HBV surface antigen, rubella virus IgG and HIV 1 and 2 antibody (HIV status assessed only with explicit consent on request form / EPR).

6 Examinations referred to other laboratories

6.1 Virus isolation referrals

Test	Sample type	Reference Laboratory	Comments
Enterovirus typing	Picornavirus isolate or original sample	Centre for Infection, HPA, Colindale	Routinely sent to characterise the type of enterovirus
HSV antiviral resistance testing by phenotyping	HSV isolate from the laboratory after cell culture of vesicle fluid / swab	Centre for Infection, HPA, Colindale	On request if clinically indicated. Isolation of HSV in cell culture is required. Contact medical staff for advice
HSV antiviral resistance testing by genotyping	HSV isolate or original sample	Centre for Infection, HPA, Colindale	On request if clinically indicated. Contact medical staff for advice
Influenza typing	Influenza isolate or original sample	Centre for Infection, HPA, Colindale	Routinely sent as part of national surveillance of the isolation of influenza

6.2 Virus serology + molecular referrals

Virus	Sample type	Laboratory method			Reference Laboratory	Comments
		IgM	IgG	PCR		
Dengue and other flaviviruses	10ml clotted blood	√	√	√	HPA CEPR, Special Pathogens Reference Unit, Porton Down	On request with relevant travel and clinical details. Contact medical staff for advice
Haemorrhagic fever viruses	10ml clotted blood	√	√	√	HPA CEPR, Special Pathogens Reference Unit, Porton Down	Contact medical staff for advice
Hantaan virus	10ml clotted blood	√	√	√	HPA CEPR, Special Pathogens Reference Unit, Porton Down	On request with relevant travel and clinical details. Contact medical staff for advice
Hepatitis E virus (HEV)	10ml clotted blood	√	√	√	Centre of Infection Colindale	On request with relevant travel and clinical details
HSV type-specific serology	10ml clotted blood		√		Centre of Infection, Colindale	On request with relevant clinical details
Measles virus IgG, IgM and RNA	10ml EDTA blood and CSF	√	√	√	Centre for Infection, Colindale	On request with relevant clinical details. Contact medical staff for advice
	Oracol saliva kit available from Health Protection Unit: 020 3049 4280	√	√	√	Sample posted by patient / clinical staff to Centre for Infection, Colindale. Within Kings, this test is available on EPR	Some kits are available in virology for local Kings Hospital use
Mumps virus IgG, IgM and RNA	10ml clotted blood	√	√	√	Centre for Infection, Colindale	On request with relevant clinical details
	Oracol saliva kit available from Health Protection Unit: 020 3049 4280	√	√	√	Sample posted by patient / clinical staff to Centre for Infection, Colindale. Within Kings, this test is available on EPR	Some kits are available in virology for local Kings Hospital use

Virus serology + molecular referrals contd..

Virus	Sample type	Laboratory method			Reference Laboratory	Comments
		IgM	IgG	PCR		
Polio	10ml clotted blood		√	√	Centre for Infection Colindale	On request with relevant clinical details
Rabies	10ml clotted blood	√	√	√	HPA CEPR, Special Pathogens Reference Unit, Porton Down	Contact medical staff for advice
West Nile Virus	10ml clotted blood	√	√	√	HPA CEPR, Special Pathogens Reference Unit, Porton Down	On request with relevant clinical details. Contact medical staff for advice

6.3 Viral nucleic acid test referrals

Test	Sample type	Reference Laboratory	Comments
Adenovirus DNA - quantitative	10ml EDTA blood	HPA - Bristol	Known adenovirus DNA positive samples only.
BK virus DNA quantitative assay in all other patients	10ml EDTA blood and urine	Centre for Infection Colindale	On request with relevant clinical details. Contact medical staff for advice
HTLV proviral DNA PCR	10ml EDTA blood	Centre for Infection Colindale	On request with relevant clinical details. Contact medical staff for advice
JCV DNA PCR	CSF	Centre for Infection Colindale	On request with relevant clinical details
Measles virus RNA PCR	10ml EDTA blood, saliva, urine, CSF	Centre for Infection Colindale	On request with relevant clinical details. Contact medical staff for advice
	Oracol saliva kit available from Health Protection Unit: 020 3049 4280	Sample posted by patient / clinical staff to Centre for Infection, Colindale. Within Kings, this test is available on EPR	Some kits are available in virology for local Kings Hospital use
Mumps virus RNA PCR	Saliva, urine, CSF.	Centre for Infection Colindale	On request with relevant clinical details. Contact medical staff for advice
	Oracol saliva kit available from Health Protection Unit: 020 3049 4280	Sample posted by patient / clinical staff to Centre for Infection, Colindale. Within Kings, this test is available on EPR	Sample posted by patient / clinical staff to Centre for Infection, Colindale
Parvovirus B19 DNA PCR	10ml clotted blood	Centre for Infection Colindale	On request with relevant clinical details. Contact medical staff for advice
Rubella virus RNA PCR	10ml EDTA blood, saliva, urine, amniotic fluid	Centre for Infection Colindale	On request with relevant clinical details. Contact medical staff for advice
HBV DNA viral load in Health Care Worker	10ml clotted blood	HPA Birmingham	On request with relevant clinical details
HIV-1 CCR5 / CXCR4 tropism assay trofile assay	2 x 10 ml EDTA blood	Lab21 (further sent to USA)	On request with relevant clinical details. Contact medical staff for advice
HIV-2 RNA load	2 x 10 ml EDTA blood	UCLH	On request with relevant clinical details. Contact medical staff for advice
HIV-2 RNA resistance	10ml clotted blood	HPA Birmingham	On request with relevant clinical details
HIV-1 antiretroviral resistance testing by phenotyping and virtual phenotyping	10ml EDTA blood	VIRCO, Belgium	On request with relevant clinical details. Contact medical staff for advice. (Turnaround time: 4 weeks)

6.4. Antiviral assays

Test	Sample type	Reference Laboratory	Comments
Acyclovir and ganciclovir plasma levels	10 ml clotted blood	Antimicrobial Reference Lab, Bristol	On request. Contact medical staff for advice with relevant clinical details

Label the specimen containers with time of previous dose.

7. Specimen collection methods

Aspirates and fluids from normally sterile sites

Collect the specimen with a sterile syringe. Transfer a maximum of 20ml into a sterile universal container. Ensure the cap is tightly screwed on.

Biopsies

All biopsies should be placed in sterile saline and not in formalin. Please state clearly the clinical diagnosis and the test needed. It may be better to send a sample for histopathology than for virology.

Bronchial washings

After collection remove the cap and the tubing of the sterile suction container and apply the screw cap to the container.

Bronchoalveolar lavage

A specialist will collect the specimen in a sterile container according to local protocol. Traps containing a specimen should be sealed using a loop of tubing.

Cerebrospinal fluid

An adequate amount is essential - send at least 2-3ml.

Cervical swabs

Chlamydia trachomatis and *Neisseria gonorrhoea*:

Take a *Chlamydia* specimen after the Microbiology swab. If there is discharge, mucus or pus in the cervix, then wipe it off. Insert the Chlamydia swab into the endocervical canal. Rotate firmly around the surface of the canal for 5-10 seconds, withdraw the swab without touching any vaginal surface. Place the swab in APTIMA sample collection tube, snip off the shaft and screw the cap on.

Neisseria gonorrhoea

This test is part of combined *Chlamydia trachomatis* and *Neisseria gonorrhoea* testing on the same sample using TMA technology by APTIMA

Virology:

Moisten the swab in sterile saline before taking the specimen. Never moisten swab in VTM. Follow procedures as for the Microbiology swab. Snap off the swab tip into VTM.

Ear swab

Place the swab in the ear canal over any vesicle. Rotate gently over the vesicles / ulcers. Place the swab in VTM.

Eye swab

Chlamydia trachomatis:

The aim is to collect epithelial cells and assess *Chlamydia trachomatis* nucleic acid by TMA. Scrape from conjunctiva and place the swab in APTIMA sample collection tube, snip off the shaft and screw the cap on.

Virology:

Moisten the swab in sterile saline before taking the specimen. Never moisten swab in VTM. Snap off the swab tip into VTM.

Faeces

Send a "plum-sized portion" or 5-10ml if liquid. Ask the patient to defecate into a clean bedpan or other convenient container if at home. Use the plastic spoon to transfer a portion of faeces into the pot. For liquid faeces use a plastic medicine spoon. Take care not to contaminate the outside of the faeces pot.

Genital tract swabs

Chlamydia trachomatis and *Neisseria gonorrhoeae*:

The aim is to collect epithelial cells and assess nucleic acid by APTIMA (TMA). Scrape from the endothelium and place the swab in APTIMA collection tube, snip off the shaft and screw the cap on.

Virology:

Moisten the swab in sterile saline before taking the specimen. Never moisten swab in VTM. Snap off the swab tip into VTM.

High vaginal swabs

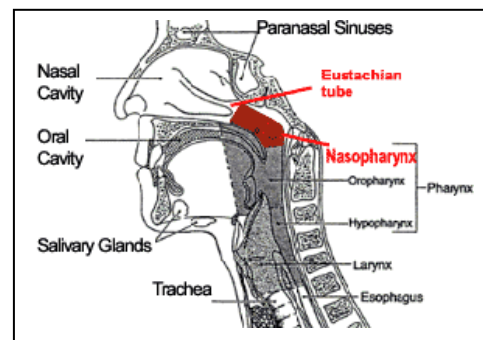
Introduce the speculum. Roll the swab firmly over the surface of the vaginal vault. Place the swab in VTM.

Mouth swabs

Sample any vesicle lesions or ulcers or inflamed areas. A tongue depressor or spatula may be helpful to aid vision and avoid contamination from other parts of the mouth. Place the swab in the VTM.

Nasopharyngeal aspirate (NPA)

Aspirate collected from the nasopharynx (see diagram) is ideal for viral upper respiratory tract infection as it contains epithelial cells, which harbour viruses.



Materials: Suction pump, sterile suction catheter (usually size 10, smaller for infants), sterile container usually 30ml urine jar, sputum pot or sterile container.

1. Attach catheter to suction pump, leaving wrapper on suction catheter. Turn on suction and adjust to a pressure of 100-150 mmHg.
2. Without applying suction, insert catheter into the nose, directed posteriorly and towards the opening of the external ear. Stop when you feel a resistance (you have reached the posterior nasopharynx).
3. Apply suction
4. Using a rotating movement, slowly withdraw catheter. Catheter should remain in nasopharynx no longer than 10 seconds.
5. Disconnect suction. Put the catheter in a sterile universal container and label.
6. Send to virology without delay. (Phone virology if you are sampling after 3pm or "out-of-hours".)

Serology

Collect 10ml of blood in a blood collection tube (usually red or gold topped). Heparinised blood may cause non-specific reactions in some antigen / IgM assays, but we can use this sample for routine serology and may lead to delays in reporting results.

Slides for immunofluorescence

NB: this is not a sensitive method and we no longer provide this service.

Sputum

Induced sputum or expectorated sputum can be used for virological assessment.

Do not collect shortly after the patient has been eating, drinking or cleaning their teeth. Ask a physiotherapist to assist if a patient has difficulty in producing satisfactory specimens.

Surface swabs and skin swabs

Rotate the swab on or in the required site. Place the swab in the VTM.

Throat swabs

Moisten the swab in sterile saline before taking the specimen. Never moisten swab in VTM. Snap off the swab tip into VTM. A combined throat and nose swab can also be taken (see above).

Throat & nasal swabs – (combined throat and nose swab)

This is the preferred to stand-alone throat swab for respiratory infections. Moisten the swab with sterile saline (never with VTM) before swabbing. Using two sterile saline moistened swabs, swab throat and nose sequentially. For nose - swab the anterior nares by gently rotating the swab in each nostril. Snap off the swab tips into VTM.

Tissues and biopsies

Under aseptic conditions transfer material to a sterile universal container that does not contain formalin as this inactivates pathogens very rapidly. Send in 0.5ml of sterile saline. Please specify which virus is being investigated for virology.

Urethral swabs

Avoid contamination with microorganisms from the vulva or the foreskin. Small swabs are available for this purpose.

Chlamydia:

Take this specimen after the Microbiology swab. Pass the swab through the urethral meatus and gently but firmly roll it over all the surfaces of the urethral epithelium for 1-2 seconds then withdraw. Snip off the swab into APTIMA sample collection tube.

Urine

Clean-voided midstream urine is preferred for virology.

It is recommended that in females the hands and the perineal area should be washed with soap and water prior to specimen collection. Part the labia and clean the area around the urethral meatus from front to back. Spread the labia with the fingers of one hand.

In males retract the foreskin, if present, and clean the skin surrounding the urethral meatus.

Start passing urine into the toilet, bedpan or urinal. When the urine is flowing freely, collect urine in a clean sterile container.

Special urine collection pouches are needed for collection in paediatric patients.

First catch urine

For *Chlamydia trachomatis* and *Neisseria gonorrhoea*, this is needed rather than mid-stream urine. The first 5 – 10 ml of voided urine is to be sent.

Vesicles, ulcers and genital lesions (see section 8)

Material required

Swabs preferably Dacron-tipped swabs with plastic shaft and viral transport medium (VTM): This is a pink fluid available from virology during normal working hours. Limited stocks may be available in the refrigerators in some wards and clinics. VTM has to be kept refrigerated and has a shelf life of 3 months at 4°C.

Method

Burst a vesicle using a sterile needle and collect with a swab or aspirate the fluid contents of the vesicle. Alternatively, scrape the base of the vesicle or ulcer with a swab so that cellular material is collected. Inoculate this fluid / cellular material into VTM.

Always state the site, distribution and nature of the vesicle. This is essential, as the laboratory may need to prioritise between HSV and VZV testing. In a case with suspected hand, foot and mouth disease we perform enterovirus RNA PCR.

8. Table of containers for transport of specimens

Specimens should be transported and processed as soon as possible

Specimen/investigation	Container and comments
Aspirates from normally sterile sites (joint, ascites, peritoneal and pleural fluids)	Sterile universal container
Biopsies	Sterile container with sterile saline.
Bronchial washings	Sterile container; e.g. 30ml sterile container or sterile universal container
Bronchoalveolar lavage	Sterile container; e.g. 30ml sterile container or sterile universal container
Cerebrospinal fluid (CSF)	Sterile universal container.
Cervical swab	For the investigation of <i>Chlamydia trachomatis</i> and <i>Neisseria gonorrhoea</i> use the APRIMA swab and the transport medium. Use swab in VTM for other viruses like HSV.
Conjunctival and corneal swabs	Use a chlamydia swab in chlamydia transport medium APTIMA for <i>Chlamydia trachomatis</i> . Use swab in VTM for virology.
Ear swab	Send a swab in VTM.
Eye swab	Use a chlamydia swab in chlamydia transport medium APTIMA for <i>Chlamydia trachomatis</i> . Use swab in VTM for virology.
Faeces	With the spatula provided transfer a plum-sized portion of faeces, or equivalent volume of fluid, into a sterile universal container
High vaginal swab (HVS)	For <i>Chlamydia trachomatis</i> and <i>Neisseria gonorrhoea</i> investigations send swab in APTIMA transport medium
Mouth swab	Send the swab from lesion in VTM.
Nasal swab	Send the swab in VTM.
Nasopharyngeal aspirate	Traps containing a specimen should be sealed using a loop of tubing
Seminal fluid	Sterile universal container
Specimen/investigation	Container and comments

Sputum	Sputum from deep expectoration and not saliva is required. Send specimen in a 30ml sputum container or universal. It is usually difficult to perform respiratory PCRs on this sample.
Throat & nasal swab	Send the swab in VTM.
Tissues and biopsies	In at least 0.5 ml of sterile saline in sterile universal container.
Urine	Collect urine in a sterile universal container. Send first catch urine for Chlamydia trachomatis.
All other urine specimens	Sterile universal container
Urethral swab	For the investigation of <i>Chlamydia trachomatis</i> and <i>Neisseria gonorrhoea</i> use the APRIMA swab and the transport medium
Vesicles, ulcers and genital lesions	Send a swab in VTM.

- In the absence of readily available VTM (laboratory issued or commercial), please immerse the swab tips in 1 ml of sterile saline in an universal container