UCD National Virus Reference Laboratory

User Manual

and Pathogen Index

Edition 29.0

December 2023

Identifier: UM-001

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Revision 29 approved by:

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Review due 1 year from Active date

Table of Amendments

Revision	**Date of Revision**
Revision No 26 Obsolete	**26, obsolete date: 16/03/2022**
Revision No 27 Obsolete	**27, active date: 16/03/2022**
Revision No 28 Obsolete	**28, active date: October 2023**
Revision 29	**29, active date: December 2023**
Sections involved	**Amendments**
New Cover Page	New cover PAGE INSERTED, old one deleted; INAB logo removed
Laboratory Address	Amend address to include the up to date Eircode D04E1W1
General Information	All References to NVRL Satellite Lab NSLB have been deleted 1.12 commencement of renal dialysis added 1.9 Contacts updated 1.14 Information included regarding verbal requests 1.15 Packing the specimen for transport – much detail removed to Appendix 1.0. Also updated to include requirements 1form, 1 sample, 1 bag, in order to avoid contamination in transit.
Referred work	2.12 Amend Leptospira Reference Lab to PHE Porton Down 2.12 Delete reference to UK PHE Colindale for HIV Resistance/Genotyping 2.12 Delete reference to Eurofins and Teagasc for SARS-CoV-2 WGS
4.0 Communication of Results	4.0 Communication section - updated 4.3 Telephoning of results - updated
5.0 Specimen Request forms and Specimen Collection	Plasma has been further identified as EDTA Plasma 5.2 Updated: removal of Forms for HIV Resistance and HIV Tropism – incorporated into LF-UM-001n, and LF-UM-001t and LF-UM-001u – both related to SARS-CoV-2 Ab studies. 5.4 Rejection list updated to include samples spilt or broken in transit and any that are contaminated, and a statement regarding samples received at NVRL in error

	5.5 Amended to include reference to Blood Tube Guide (LF-UM-001w) and to Swab Guide (LF-UM-001x) found on NVRL website. Also information included about correct use of Aptima® devices
6.0 Investigation of Suspected Congenital Infection	6.3 Amended in line with CN-22-6 6.4 Dried Blood Spot - updated
6.6 Travel Related Infections	6.6 Reference to LF-UM-001d – VHF Request form
6.10 Blood borne virus (BBV) screening	6.10.4 amended to include reference to Risk Bulletin CN-22-22
7.0 Reference Ranges	Amended to include description and links to national guidelines
8.0 NVRL Pathogen Index	Details regarding the Virus Description pages
9.0 Viral Taxonomy	Details regarding Viral Taxonomy are included here
Virus Description Paragraphs - Pages 29-end	Pg 29: Adenovirus - updated Pg 35: Lyme Neuroborreliosis requires 600µl CSF. Reference also to RIPL User Manual Pg 46: Enterovirus paragraph updated Pg 53: HAV PCR testing is done weekly Pg 54: HBV paragraph updated Pg 56: HCV Genotyping not done if previously tested within 6 months Pg 59 and 60: HHV-7 and HHV-8 – updated to include EDTA PLASMA as preferred sample type Pg 62: HIV-2 EDTA plasma and EDTA whole blood are acceptable Pg 69: Influenza paragraph updated Pg 75: Reference laboratory amended to Porton Down Pg 78: Amended to read: No NVRL testing is available Pg 81: Monkeypox Virus Pg 84: Mycoplasma genitalium updated Pg 105: SARS-CoV-2 Antibody - these tests are carried out on request Pg 114: T pallidum – sample volumes included relating to the diagnosis of Congenital infection; TPPA replaced by TPHA Pg 127: West Nile Virus – update to NVRL tests
Insert Appendix	Appendix inserted in relation to **Packing of Specimen for Transport** – previous 2.15.1 and 2.15.2

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1. INTRODUCTION

This manual is designed to give an overview of the services provided by UCD National Virus Reference Laboratory (NVRL). The laboratory provides a national diagnostic, reference, and clinical liaison virology service for clinicians investigating virus infections. The laboratory is accredited by the World Health Organisation (WHO) as a National Laboratory for Poliovirus, Influenza, Measles and Rubella. An out of hours service is available 365 days a year to deal with urgent testing requirements, such as screening of solid organ donors, testing of those requiring renal dialysis and investigation of sources of needle-stick injuries. Requests for this service must be approved at the requesting hospital by the relevant Consultant Microbiologist / Pathologist and by the NVRL on-call clinician. A wide range of diagnostic tests is performed routinely but others are available by prior arrangement. The tests available are outlined in this document and in the associated Pathogen Index.

Dr Cillian De Gascun
DECEMBER 2023

GENERAL INFORMATION

1.1 Laboratory Address

National Virus Reference Laboratory
University College Dublin
Belfield
Dublin 4
Ireland

1.2 D04 E1W1General NVRL Contact Details

General Telephone:	+353 - 1 - 716 4401
Fax:	+353 - 1- 269 7611
E-mail:	nvrl@ucd.ie
Website:	nvrl.ucd.ie
Customer Care Officer	helen.dawkins@ucd.ie; +353 -1-716 1257

1.3 Result line +353 -1- 716 4414

Monday to Friday: 08.30 – 13.00; 14.00 – 17.30 hrs

1.4 Laboratory Opening Hours:

Monday to Friday: 08.00 - 18.00 hrs.

1.5 Clinical Liaison Service

The National Virus Reference Laboratory (NVRL) provides a clinical liaison service to users, the role of which is to advise on the appropriate investigations for, and management of, specific viral infections.

The clinical liaison service is provided by the clinical team within the NVRL and is available both during regular working hours and out-of-hours

The clinical team currently consists of a consultant medical virologist, two consultant medical microbiologists, one principal clinical scientists, and two senior clinical scientists. Contact details for the clinical team are provided in section 2.9 of the User Manual.

1.6 Laboratory Accreditation

The NVRL is accredited by the Irish National Accreditation Board (INAB) to undertake testing as detailed in the Schedule bearing the Registration Number 326MT, in compliance with the International Standard ISO/IEC 15189:2012. For the most recent accreditation certificate please see the NVRL website nvrl.ucd.ie

1.7 Out of Hours Service

An "out of hours service" is provided by the NVRL for urgent laboratory investigations. Please note that – to ensure continuity of patient care – the Consultant Microbiologist/ Pathologist at the requesting hospital should be involved in the decision to request out of hours testing.

The NVRL On-Call Clinical Team must also approve any request for investigation (as not all tests are available out of hours). The on-call service can be accessed outside normal hours by contacting 01 7164050. When using this number, the following information is required:

a) Name of caller
b) Contact number
c) Hospital source
d) Message

N.B. Results will be telephoned to the *requesting* doctor. To ensure prompt communication of results, it is ESSENTIAL that the request form accompanying the specimen specifies the requesting doctor's name and contact number (mobile number if possible).

Organ Donation and Transplant Ireland

The NVRL performs donor screening as part of the National Organ Procurement Service for Ireland (ODTI). This service is provided with the assistance of procurement coordinators that cover individual hospitals. To access this service, please contact the Clinical Team at NVRL – see the list of contacts below. Out of hours, please contact 01-7164050 and a member of the On-Call Clinical team will respond.

1.8 List of Contacts

Telephone enquiries for patient results are available between 08.30 – 13.00hrs and 14.00 - 17.30hrs Monday-Friday. The telephone numbers to contact are outlined below. Results are reported (hard copy and in many cases electronically) as soon as they are authorised.

Please note that many reports are issued directly to the hospital pathologist/microbiologist: therefore, it is advisable to check with your pathology department before calling the NVRL. Please also consult the stated test turnaround time (see Virus Testing Index, LF UM 001r, on https://nvrl.ucd.ie) to ascertain when the result is likely to be available.

Any queries relating to service provision or the User Manual should be directed to the Laboratory Manager, the Customer Care Officer or the Quality Assurance Officer. Requests for Medical Advice regarding diagnosis and treatment of infection should be directed to a member of the Clinical Team (01-716 4401). Additional contact numbers are provided in the Table below.

	Name	Phone Number
Director Consultant Virologist	Dr Cillian De Gascun	716 1223
Deputy Director	Dr Daniel Hare	716 1223
Laboratory Manager	Deirdre Burke	716 1328
Assistant Director & Principal Clinical Scientist	Dr Jeff Connell	716 1321
Consultant Microbiologist with Special Interest in Virology (SJH)	Dr Brendan Crowley	416 2968
Senior Clinical Scientist: Molecular Virology	Dr Jonathan Dean	716 1253
Senior Clinical Scientist: Serology	Paul Holder	716 1347
Quality Assurance Officer	Eimear Malone	716 4400
Systems Manager IT & Communications	Brian O'Grady	716 1346
Facilities Manager	Geoffrey Gray	716 1235
Finance Officer	Michelle Keogh	716 4403
Surveillance Scientist	nvrl.surveillance@healthmail.ie	
Customer Care Officer	Helen Dawkins	716 1257
Results line 08.30 – 13.00, 14.00 - 17.30hrs		716 4414
Clinical line 09.00 – 13.00, 14.00 – 17.30hrs		716 4414
Urgent Laboratory Investigations		716 4401

1.9 Complaints & Compliments

Please contact the Customer Care Officer (CCO): Feedback from our users – both positive & negative – allows us to continually refine and improve the service we provide.
The Customer Care Officer will log a record of your feedback on the NVRL Quality Management System – QPulse - and will assign a unique identifier to your compliment/complaint. Your experience will be discussed and corrective action will be applied if merited. The CCO will be in touch with you by phone or by email, as appropriate, in order to apply Corrective Action, and hopefully resolve any and all complaints to mutual satisfaction. NVRL always strives to ensure the quality of service provided meets or exceeds our INAB requirements.
Compliments on NVRL service provision are also welcome. A Customer Feedback Form is being drafted for the NVRL website

1.10 Laboratory Fees

A list of standard charges, which is updated annually, is provided to customers. Please note that an additional charge *may* be levied on out-of-hours services. Telephone inquiries can also be made to 01 716 4403

1.11 Referred Work

For the purposes of additional or confirmatory investigations, samples may be referred to an external laboratory (see list below):

Pathogen	***External Laboratory***	***Accreditation***
Rickettsiae Arthropod borne viruses Haemorrhagic fever viruses *Hantavirus*	Rare and Imported Pathogens Laboratory (RIPL) PHE Microbiology Services Porton Down Salisbury Wiltshire SP4 0JG	UKAS
Toxoplasma gondii (Toxoplasma referrals occur based on clinical details and initial results obtained in the NVRL)	Toxoplasma Reference Laboratory PHE Swansea Singleton Hospital Sketty Swansea SA2 8QA	UKAS
Leptospira icterohaemorrhagiae (Leptospira referrals occur based on clinical details and initial results obtained in the NVRL)	Rare and Imported Pathogens Laboratory (RIPL) PHE Microbiology Services Porton Down Salisbury Wiltshire SP4 0JG	UKAS
Borrelia burgdorferi (Lyme Borreliosis referrals occur based on clinical details, sample type and initial results obtained in the NVRL.)	Rare and Imported Pathogens Laboratory (RIPL) PHE Microbiology Services Porton Down Salisbury Wiltshire SP4 0JG	UKAS
JC Ab Hepatitis B Virus: HBsAg quantification testing.	Virus Reference Department Microbiology Services PHE Colindale 61 Colindale Ave London NW9 5HT	UKAS
Chlamydia trachomatis (Rectal swab positive samples are referred for LGV Typing) LGV Testing	Bacteriology Reference Department, PHE Colindale 61 Colindale Ave London NW9 5HT	INAB

HIV 2 RNA Testing is not available in the NVRL so it is *essential* samples are correctly labelled as HIV-2 (and not just HIV) ***Separated EDTA plasma or Whole EDTA blood***	Clinical Microbiology and Virology UCL NHS Foundation Trust 5th Floor Central 250 Euston Road London NW1 2PG	UKAS
Mycoplasma genitalium	PHE Colindale 61 Colindale Ave London NW9 5HT	**UKAS**
HDV Viral Load	EUROFINS BIOMNIS 17-19 AV TONY GARNIER 69007 LYON 7EME SIREN N° 493519904 France	**COFRAC**

1.12 Urgent Laboratory Investigation

To aid the NVRL in identifying specimens that are deemed clinically urgent, users are requested to follow the urgent testing protocol below.

Please note: due to the number of specimens received in the NVRL daily, it is ***not*** sufficient to simply write urgent on the request form.

1: Contact the NVRL prior to sending the sample
If a sample is deemed urgent please contact the NVRL clinical team PRIOR to sending the sample (01 716 4401). It takes considerable time and effort to retrieve & identify samples that have been received in the NVRL without advance notification. The NVRL maintains an Urgent Tracking System (UTS) for managing urgent referred samples.

2: Provide the name and contact number (mobile if possible) of requesting clinician
It is ESSENTIAL that these contact details are accurate and a designated person is available to discuss the request as further information may be required before the sample is processed as urgent.

3: Ensure urgent samples are clearly identified particularly when they are included in routine delivery boxes (which can contain hundreds of samples)
Urgent samples should be packaged in a separate container/envelope and clearly marked as URGENT on the external packaging and request form.

4: Result reporting
It is ESSENTIAL that contact details are provided for results to be telephoned when required. Please be aware that results of certain assays may not be available until after 17.00 hrs., therefore, contact details for out of hours reporting should be provided on all samples considered clinically urgent.

NB: THE ONUS REMAINS ON THE REQUESTING CLINICIAN TO FOLLOW UP ON RESULTS FROM SAMPLES THAT ARE DEEMED CLINICALLY URGENT.

'Routine' Urgent Tests

During regular working hours, and where appropriate clinical details are provided on the accompanying test request form, the tests below will be performed urgently as a matter of course.

- Source of a needle-stick
- Organ donor
- Un-booked woman in labour (or who has just delivered) in the absence of booking bloods
- Patient requiring sperm banking.
- Pregnant woman in contact with Varicella Zoster virus
- Commencement of renal dialysis

NB: IF THE ABOVE TESTS ARE REQUIRED OUT OF HOURS, THE ON CALL MEMBER OF THE NVRL CLINICAL TEAM MUST BE NOTIFIED – contact 01 7164050, including Name of Caller, Contact number, Hospital source and Message

1.13 Additional Laboratory Testing

If **additional laboratory testing** is required by the referring clinician on a sample that has already been received in the NVRL, the NVRL may be able to facilitate this additional test request without the need for re-bleeding or recalling the patient. The laboratory can be contacted (01 716 4401) to investigate the feasibility of using the initial specimen for analysis. Additional testing can be performed on receipt of a written/faxed request or through the NVRL website nvrl.ucd.ie. Verbal requests for additional testing cannot be accepted. All requests for additional testing should be submitted to NVRL via the website: https://nvrl.ucd.ie/additional_testing , or should be received via email/healthmail, ensuring as far as possible that patient details are provided appropriate to the email. (ucd.ie addresses are not encrypted - please do not include more than 1 patient identifier)

1.14 Packing the Specimen for Transport

The requirements stated below are in accordance with the European Agreement concerning the International Carriage of Dangerous Goods by Road (UNADR) and apply to all specimens or samples directed to the NVRL. Infectious substances are classified in Division 6.2 and assigned to UN 2814, UN 2900, UN 3291 or UN 3373, as appropriate. It is the responsibility of the referring site to ensure compliance with these requirements.

Infectious substances are divided into two categories, Category A and Category B.

NOTE: Majority of specimens sent to NVRL are considered Category B. Please go directly to Appendix 1.0 Procedure for the Transport of Infectious Substance Category A and Category B.

General preparation of shipments for transport
Because of the differences in the hazards posed by Category A infectious substances (UN 2814 and UN 2900) and Category B infectious substances (UN 3373), there are variations in the packaging, labelling and documentation requirements for the two categories. The packaging requirements are determined by UNCETDG and are set out as Packing Instructions P620 and P650. The requirements are subject to change and regular upgrade by the organizations mentioned. The current packaging requirements are described below.
Shippers of infectious substances shall ensure that packages are prepared in such a manner that they arrive at their destination in good condition and present no hazard to persons or animals during transport.
Please go to Appendix 1.0 for comprehensive information on packing and transport of samples.

NVRL has specific requirements regarding receipt of samples, which will mitigate the need to reject samples for testing. Samples should arrive individually packaged – ie 1 form, 1 sample, 1 bag. This will increase the amount of packaging required for a delivery. The benefit is that a spillage or breakage in transit will not affect any other sample. Thus, only 1 sample will be rejected.

2. ASSESSMENT OF A PATIENT WITH SUSPECTED VHF INFECTION OR NOVEL ZOONOTIC INFECTION – SUCH AS MONKEYPOX VIRUS INFECTION (HMI)

Patients suspected of a VHF/HMI infection are assessed using the Clinical Risk Assessment Form http://www.hpsc.ie/a-z/vectorborne/viralhaemorrhagicfever/guidance/vhfforms/

Following the completion of this form, patients will be categorised as either:
• no risk
• possibility of VHF (***At Risk***)
• high possibility of VHF (***High Risk***)
If an Irish hospital or clinical laboratory is concerned a patient may have potential VHF exposure they MUST CONTACT the National Isolation Unit, Mater Hospital, Dublin, the NVRL and the relevant Public Health Department who will advise if the patient is at risk of VHF infection based on the completed Clinical Risk Assessment Form.

2.1 Collection of clinical specimens from patients with suspected or confirmed VHF.

Only specimens essential for diagnosis or monitoring should be obtained for VHF investigation. Appropriate PPE must be worn during specimen collection (see http://www.hpsc.ie/a-

z/vectorborne/viralhaemorrhagicfever/guidance). Standard Precautions should be applied when sampling from a patient categorised as ***At Risk***. If the patient is symptomatic then Standard plus Contact plus Droplet Precautions should be applied. This should be increased to Standard plus Contact plus Droplet plus Airborne Precautions when sampling from ***High Risk***.
The preferred specimen for diagnosis of VHF virus is EDTA blood. Ideally, 5ml EDTA blood is required to complete the VHF analysis although 1ml volume is sufficient to perform essential tests where sufficient sample volume is difficult to obtain (i.e. infants and young children). Urine and post-mortem tissue samples are also suitable for testing. EDTA Plasma samples for molecular investigation should be separated from whole blood within 24 hours of venepuncture and frozen immediately at -20°C to maintain the integrity of the viral genetic material.
Please note that specimens anti-coagulated with heparin/sodium citrate are not suitable for PCR.

2.2 Human Monkeypox infections

These are managed as per the guidance found on the Health Protection Surveillence Centre (HPSC) website (see link below). Swabs should be sent as per transport for Category A pathogens. All other samples from the suspected case should be held in the source laboratory until the PCR testing is complete and a 'not detected' result is available. Only then can other samples – blood, Aptimas, stools etc – be safely transported to NVRL as Category B samples. Further diagnostic work-ups on patients with a 'detected' result can be discussed with NVRL Clinical team.
https://www.hpsc.ie/a-z/zoonotic/monkeypox/guidance/Laboratory%20transport%20plan.pdf

3. SPECIMEN RETENTION POLICY

In accordance with the guidelines of the Royal College of Pathologists (UK), the NVRL will retain all serum/plasma specimens for 4 months following issue of final report unless otherwise arranged. There are some exceptions to this policy:

- Antenatal samples (retained for 2 years)
- Serum taken after needle-stick injury or otherwise hazardous exposure (retained for 2 years)
- Genital swabs (retained for 1 month)
- BTE samples (retained for 30 years)
- National Organ Procurement Service for Ireland samples (retained for 11 years post transplantation)
- Post mortem and biopsy material (retained indefinitely)
- Samples from unknown sources (retained for 1 month)

- Samples sent in Aptima Collection devices for Chlamydia/Gonorrhea, Trichomonas vaginalis and Mycoplasma *genitalium* are retained for one month

4. COMMUNICATION OF RESULTS

The NVRL issues paper reports by arrangement to the requesting clinician. Reports are generally securely transmitted electronically via Medibridge/ Healthlink to hospital laboratories or GPs respectively. Please contact the NVRL if you would like further information on the electronic communication of results.

For reasons of confidentiality **it is the policy of the NVRL not to e-mail reports**. In addition, results are not routinely transmitted by facsimile. However, if the NVRL can be assured of the security of the recipient's facsimile, individual reports can be faxed if necessary or sent via healthmail from nvrl.admin@healthmail.ie or nvrl.qa@healthmail.ie

It is the policy of the NVRL to comply with the Data Protection Acts 1988 and 2003 in relation to all personal information received in the course of processing specimens.

Delays with results, relating to technical issues will be communicated via the NVRL website (https://nvrl.ucd.ie) as Customer Notifications.

Users are asked to not include patient information in un-encrypted emails, as per NVRL GDPR guidelines. N.B.: ucd.ie addresses are not secure. Please use Healthmail if querying patient samples or results.

4.1 Unexpected Results

The NVRL reviews all results generated and utilises IT solutions to detect – insofar as is possible – inconsistent or discordant patient results obtained over time. However, the absence of a unique patient identifier means that this system is not perfect.

As such, the NVRL strongly recommends that users send repeat samples from patients where unexpected or surprising results have been received, or when requested by the NVRL.

4.2 Copy Reports

Please note that patient consent is required if the copy report is not going to the source of the original request. Copy reports and patient consents (LF UM 001q) and additional test requests are available on the website: nvrl.ucd.ie (https://nvrl.ucd.ie/copy_results) or via fax request to 01-2697611.

4.3 Telephone Results

The NVRL regrets that it is not in a position to routinely communicate results by telephone. However, the laboratory does appreciate that in certain circumstances, telephone communication

of significant results may be indicated. As such, the laboratory has implemented a policy whereby – in addition to Urgent Laboratory Investigation samples (Section 2.11) – the following time-sensitive, demonstrably abnormal, results will be communicated to the requesting clinician or laboratory:

Molecular
- CSF samples testing positive for viral RNA/DNA

- Significant RNA/DNA levels (>3 log copies/ml) of Adenovirus, CMV in blood samples from patients not previously known to be positive

- Any samples testing positive for CMV or HSV DNA or Enterovirus RNA in neonates

Serology
- New diagnoses of acute HIV

- New diagnoses of acute Hepatitis B

- New diagnosis of acute Hepatitis A or E in hospitalised patients

- New diagnoses of primary CMV, Parvovirus B19, T. gondii, or T. pallidum infection in pregnancy

NB: THE ABOVE RESULTS ARE ROUTINELY COMMUNICATED BY TELEPHONE: OTHER RESULTS MAY BE TELEPHONED BY ARRANGEMENT BASED ON CLINICAL NEED

5. SPECIMEN COLLECTION INFORMATION

5.1 Essential Information

A minimum of two patient identifiers e.g. Full Name AND date of birth is required on the specimen container for sample processing.

The following essential information should be documented in a legible manner on test request forms & specimen containers sent to the NVRL:

- Patient identification, including gender, date of birth, patient address and a unique identifier (e.g. hospital number)
- Specimen Type / include site of swab, tissue or other specimen
- Clinical details including symptoms and date of onset
- Investigation(s) required
- Clinician name and contact details for report destination
- Date and time of specimen collection

All of the above details are mandatory when completing request forms and requesting tests on twins or patients with the same surname. Addressograph labels are preferred. Handwritten

request forms must be legible and tests required should be clearly distinguishable. Electronic requesting is preferred as this significantly reduces errors due to misinterpreted information. Please contact our Customer Care Officer for further details (01-7161257)

5.2 Request Forms (https://nvrl.ucd.ie/info)

The NVRL accepts most hospital test request forms as long as the appropriate patient identifiers are provided*. However, the laboratory also produces its own request forms that you may find helpful. The following NVRL request forms may be downloaded from the NVRL website:

- General Virology Request Form LF UM 001b
- Arbovirus/Viral Haemorrhagic Fever Investigation Request Form LF UM 001d
- Oral Fluid Investigation Request Form LF UM 001m
- Blood Borne Virus Investigation Request Form LF UM 001n
- STI Investigation Request Form LF UM 001o
- SARS-CoV-2 RNA/COVID-19 Request Form LF UM 001s
- SARS-CoV-2 Antibody Request Form LF UM 001v
- Hepatitis of Unknown Aetiology Request Form LF-UM-001y

*** Please note:** *By submitting any request form (NVRL or otherwise) it is presumed that the consent of the patient was obtained at the time of sample collection for the testing process to be engaged.*

5.3 Samples from Private Patients

The primary role of the NVRL is to provide a clinical, diagnostic, and reference virology service on behalf of the Irish Health Service and public patients. Specimens from private patients should be identifiable so the public purse is not inadvertently charged for the testing of private samples.

5.4 Specimen Rejection & Factors that may affect Assay Performance

Specimens are rejected at specimen reception in the following circumstances:

- When the information on the form and on the specimen do not match, e.g. discordant surname, or date of birth. In this situation, the specimen will be stored in the NVRL and a report will be issued to the requesting clinician informing them of the discordance. Upon receipt of written clarification confirming the correct details on headed paper from the requesting clinician, the specimen will be processed
- Specimens that arrive at NVRL without a request form – these are stored for 1 month before discard

- When the sample is unlabelled: these samples cannot be tested as the provenance of the sample cannot be guaranteed

- If the incorrect specimen type has been received, insufficient specimen volume has been provided, or if specimen collection devices have gone beyond their expiration date: these samples cannot be tested

- Use of incorrect blood collection tubes may impact on assay performance e.g. those containing z clot activators may impact on HBV DNA (viral load) testing: these samples will be tested but a result may not be generated

- Specimens accompanied by forms requesting 'Viral Studies" are not routinely processed: clinical details are essential to ensure appropriate tests are performed

- Assay performance may be affected by sample quality: blood specimens that are grossly haemolysed, icteric (high bilirubin content) or lipaemic may not be suitable for testing

- For RNA viruses, if screening only by molecular methodologies there is an inherent risk for any RT- PCR based test system that accumulation of mutations over time may lead to false negative results

- Results can be affected by timing of sample collection relative to infection

- Incorrect levels in the Aptima Urine Collection Device, Aptima samples that have leaked or are in a container containing another leaked sample. These samples will not be tested.

- Aptima samples where the Aptima Collection device has expired

- Specimens that have arrived at NVRL unsuitable as a result of a handling error at source

- Specimen that have broken or spilt in transit, or been contaminated following a breakage or spillage of a sample in the same delivery

- Specimens that are submitted to the NVRL in error are logged on the NVRL LIMS, stored appropriately, and a report with these details is issued to the source. This NVRL report is the means of informing a source that a sample was received at NVRL in error. The onus is on the source to contact NVRL in writing if the sample is to be released for testing in another laboratory or returned to source.

5.5 Specimen Collection

The following information should be considered a general guide only: for pathogen-specific requirements, please refer to the NVRL Virus Testing Index on our website https://nvrl.ucd.ie/virus_testing_index and the Pathogen Index in Section 8 ~~(from pg 31).~~

Cerebrospinal Fluid

If possible, collect 500µl of undiluted CSF into a sterile container for molecular investigation, minimum 150µl. Transport medium is not required. Specimens should be transported without delay at +4°C.
CSF samples taken over the weekend period should be stored frozen, at source, at -20°C to maintain the integrity of the viral genetic material.
CSF that is blood stained should be centrifuged at source to remove RBCs before freezing or transport to NVRL. **This centrifugation step should be notified on the accompanying form**.

Please note: to ensure prompt processing of CSF samples, they should be transported overnight Sunday or early Monday morning to reach NVRL by 09.00 hrs Monday (09.00 hrs Tuesday in Bank Holiday weekends) in order to guarantee same day testing.

Blood Samples – for acceptable blood tubes, please view the NVRL Tube Guide (LF-UM-001w) on the website under Lab Information: https://nvrl.ucd.ie/sites/default/files/uploads/pdfs/NVRL Tube_Guide_for_Sample_Collection.pdf
It is preferable that blood tubes are filled to their stated capacity. This minimises the risk of insufficient volume for completion of testing. The NVRL will endeavour to maximise the use of any sample. In cases where sample collection is difficult or the volume collected is small please indicate the tests that are of highest priority.

Clotted Blood/ Serum

Minimum volumes	Clotted Blood	Separated Serum
Routine testing	5ml	2ml
Pediatrics	2ml	1ml

For serological investigations serum samples or a container of clotted blood should be sent to the NVRL. At a minimum, 5ml of clotted blood (2ml for paediatric samples) or 2ml of serum (1ml for paediatric samples) is required for testing. Blood collected by venepuncture should be allowed to clot. Care should be taken to ensure that the blood samples are fully clotted prior to storage or transport at +2 to 8°C. Samples not required for testing within 72 hours should be removed from the clot and stored frozen (at Minus 15ºC or colder).

Serum samples required for molecular investigations should be separated from whole blood within 24 hours of venepuncture and frozen immediately at -20°C to maintain the integrity of the viral genetic material. These samples should be transported to the NVRL in a frozen state.

EDTA Whole Blood/Plasma
At a minimum, 5ml of EDTA whole blood (2ml for paediatric samples) or 2ml of plasma (1ml for paediatric samples) is required for testing.

Plasma samples for molecular investigation should be separated from whole blood within 24 hours of venepuncture and frozen immediately at -20°C to maintain the integrity of the viral genetic material. These samples should be transported to the NVRL in a frozen state.
Blood samples received for molecular investigations must be centrifuged, separated and frozen within 24 hours of venepuncture. If samples cannot be processed accordingly at the source the

specimens should arrive to the NVRL no later than 3pm on the day of sample collection. Gel separation tubes are the device of choice for all molecular investigations to allow for easy separation of serum/plasma.

Minimum volumes	Clotted Blood	Separated Plasma
Routine testing	5ml	2ml
Pediatrics	2ml	1ml

***Please note that specimens anti-coagulated with heparin are not suitable for PCR.**

Oral Fluid

Oral fluid specimens should be collected using a OraCol™ collection device supplied by the NVRL. These devices are designed to be used in a similar way to a toothbrush. Instructions for sample collection are detailed on the packaging of the device. Please contact the laboratory for further information.

Respiratory Secretions

Respiratory viruses are extremely thermolabile and therefore should be transported to the laboratory at +4°C without delay. The quality of the sample is a major determinant in identifying the causative agent. Secretory specimens are therefore the specimens of choice.

Nasopharyngeal Aspirate

Nasopharyngeal secretions should be aspirated into a sterile plastic mucous extractor, which is then transported (with the secretions) to the NVRL. At a minimum, 1 ml of sample is required for testing

Bronchoalveolar lavage

A bronchoalveolar lavage (BAL) should be transported to the laboratory in a sterile container. At a minimum, 1 ml of sample is required for testing.

Sputum

Sputum samples should be transported to the laboratory in a sterile container. At a minimum, 1 ml of sample is required for testing.

Stool

Approximately, 5g/5ml of stool samples should be transported in a sterile universal container. Transport medium is not required. For molecular detection of viruses associated with gastroenteritis, specimens should be transported to the laboratory as soon as possible post collection. Alternatively, specimens may be stored at +4°C for up to 72hrs before dispatch.

Swabs: for acceptable swab types, please view the NVRL Swab Guide, LF-UM-001x on the website under Lab Information: https://nvrl.ucd.ie/

Once the affected site has been swabbed, the swab should be broken into viral transport medium and transported to the NVRL at +4°C. alternatively the swabs can be sent in the collection device. Please note dry swabs (no medium) will not be processed.

Urine

Approximately 2mls of urine should be sent in a sterile container. Specimens should be transported without delay at +4°C.

Chlamydia trachomatis/ Neisseria gonorrhoeae samples
Only specimens collected in **APTIMA collection devices (ACDs)** can be tested in the NVRL. Instructions for sample collection are detailed on the packaging of the device, or can be viewed on the NVRL website under Lab Information>Sample Guides at https://nvrl.ucd.ie/ a

Please note it is **ESSENTIAL** to ensure urine specimen containers are filled to the correct volume as indicated by the black lines on the SCD. Over- or under-filled SCD will not be processed in line with manufacturer recommendations as it may impact on the sensitivity of the test.
Expired collection devices may be processed under strict conditions, following advice from the manufacturer (Hologic™). Please always check expiry dates before using ACDs.

Information for Medical Professionals and Patients regarding Collection Devices.

Sample collection guides are available on our website – https://nvrl.ucd.ie/swabs - regarding collection devices that are suitable for self-collection.
These include:
STI infections: Hologic Aptima Collection Devices (ACDs) for Urine, Multitest Swabs and Rectal Swabs
Enteric Infections: Stool and Rectal Swab Sample guides
Measles/Mumps/Rubella: Oracol Saliva Collection guide

6. TESTING IN SPECIAL CIRCUMSTANCES:

6.1 Post Mortem (PM) Specimens

The NVRL accepts post mortem serum and tissue samples, but it is important to realise that the majority of commercial assays used in this situation have not been validated for PM use.

In addition, the sensitivity of molecular assays raises the possibility of identifying a viral pathogen that is not actually implicated as a cause of death.

As such, it is vital that the NVRL be contacted (ideally in advance) about PM samples to ensure that the samples can be investigated promptly and appropriately, generating usable results.

As a general rule, PM tissue samples will be placed in cell culture for viruses (See Table 6.1 Below). This approach has the advantage of being non-specific ('catch-all'), while demonstrating the presence of viable ('live') virus if it yields a positive result.

Specific molecular (PCR) testing is best performed in conjunction with the pathologist when a particular pathogen is suspected: molecular testing is far more sensitive than culture, but it does not distinguish between viable and non-viable virus.

On completion of the testing process, the NVRL will arrange for retention, disposal, or return of the unused specimens as instructed by the referring clinician or institution.

Sample Type	Viral Culture	Molecular Tests
Nasopharyngeal Aspirate	Yes	Adenovirus Enterovirus Parechovirus CMV Respiratory viruses
Meconium	Yes	Adenovirus Enterovirus Parechovirus
Swabs e.g. placenta spleen, lung	Yes	Adenovirus Enterovirus Parechovirus CMV Respiratory viruses (lung/throat/trachea swabs)
Serum	No	Tests Selected on basis of information provided
Tissue (Fresh)	Yes	Adenovirus Enterovirus Parechovirus CMV Additional Tests on the basis of information provided
Tissue (Formalin Fixed, Paraffin Embedded)	No	Adenovirus Enterovirus Parechovirus CMV Additional tests on the basis of information provided

Table 6.1: Investigation of Specimens from Post Mortem & Intrauterine Death

6.2 Intrauterine Death

The Royal College of Physicians of Ireland (RCPI) Institute of Obstetricians & Gynaecologists has published a Clinical Practice Guideline on the Investigation & Management of Late Foetal Intrauterine Death and Stillbirth, a copy of which can be found in the Resources section of the NVRL website.

PM samples from IUD & Stillbirth are processed in accordance with Section/Table 6.1 above. Investigation for Parvovirus B19, *Toxoplasma gondii*, Rubella and *T pallidum* is not routinely performed in the absence of positive maternal serology and/or relevant clinical findings.

Please note the NVRL stores antenatal bloods for two years (from the date of the sample) and retrospective testing of maternal samples may be possible.

Respiratory PCR is also carried out on some Post Mortem and IUD samples as per NVRL protocols. see Table 6.1)

6.3 Investigation of Suspected Congenital Infection

Congenital (or neonatal) infections for which the NVRL provides a diagnostic service include Toxoplasma, Rubella, CMV, Herpes, and Treponema pallidum (syphilis). However, the preferred sample type differs for some of these pathogens.

Serum is suitable for testing for Toxoplasma, *Treponema pallidum*, and rubella: however, these tests in the neonate should be performed in conjunction with maternal serology, because not all infants will produce IgM in the first weeks of life. As such, documenting the absence of IgG in the mother, or indeed documenting seroconversion during pregnancy, is a useful way of confirming/excluding infection in the neonate.
If congenital rubella is suspected, the NVRL should be contacted to discuss the most appropriate samples to collect and the most appropriate investigations to be performed.

Urine is the specimen of choice for CMV testing, with PCR for viral DNA the preferred testing methodology. Salivary fluid may also be tested for CMV DNA if attempts at capturing a urine sample are not successful. Please note that diagnosis of congenital CMV infection requires detection of virus in a sample from the child within the first 3 weeks of life. (See also the section on Guthrie Card Testing in Section 6.4)

Molecular testing (PCR) is also the preferred methodology for the diagnosis of neonatal HSV infection. Viral swabs should constitute the initial screen in the form of orolabial, conjunctival, and rectal swabs. If the child is very unwell, and there is a high index of suspicion for HSV, then ideally EDTA blood and CSF should also be collected before antiviral therapy is commenced. In this situation, maternal HSV serology may provide additional information. However, neither positive nor negative IgG in the mother excludes HSV infection in the neonate (IgG may take 2-3 months to develop following primary infection).

6.4 Dried Blood Spot/DBS (Guthrie Card) Testing

As mentioned in Section 6.3 above, the diagnosis of congenital CMV (cCMV) infection requires isolation of virus from the neonate within the first three weeks of life. If, however, testing within the first 3 weeks of life has not been performed, then the Guthrie Card/Dried Blood Spot (Heel-prick) may be tested retrospectively.

The National Newborn Screening Programme is based at CHI@Temple Street. Requests for DBS testing should be sent to CHI@Temple Street, and the DBS/Guthrie Card will be transferred from there to the NVRL for CMV IgM & DNA testing. It should be noted that DBS is not the specimen of choice for the diagnosis of cCMV as not all congenitally infected infants

will be DNA &/or IgM positive. Urine or saliva within the first 3 weeks of life is the specimen of choice. Results will be returned to CHI@Temple St and not the requesting clinician. CHI@Temple St will ensure that the NVRL report is forwarded to the requesting clinician.

6.5 Amniotic Fluid

The testing of amniotic fluid may be indicated during pregnancy if CMV, Parvovirus, or Toxoplasma infection is suspected. CMV and Parvovirus DNA testing are performed in the NVRL; specimens for *T.gondii* PCR are sent to the Toxoplasma Reference Laboratory in Swansea.https://phw.nhs.wales/services-and-teams/reference-laboratories-and-specialist-services/toxoplasma-reference-unit/ Of note, the excretion of CMV by the foetus into amniotic fluid requires functioning kidneys and as such, should not be performed until after 21-22 weeks gestation. Parvovirus DNA testing can also be performed on foetal blood at the time of cordocentesis (to confirm presence of viremia).

6.6 Travel related Infections

The NVRL provides a range of diagnostic tests (see Section 8) for pathogens that are likely to be acquired overseas. However, ensuing the appropriate use of these tests requires a comprehensive recent travel history. Nonspecific details, e.g. 'foreign travel', may result in a delayed or missed diagnosis for the patient, and an unnecessary waste of resources for the requesting institution and the laboratory.

Dates & duration of travel, regions/countries visited, and main symptoms, should be provided where feasible: a member of the clinical team is always available to discuss the most appropriate tests if you are uncertain what to request. In addition, information regarding the nature of the travel (urban/rural), and/or involvement in any particular past-times or occupations, e.g. farming, while overseas should also be provided. Please see section 2.16 in connection with Viral haemorrhagic fever (VHF) and Human monkeypox infection (HMI) testing.
A specific NVRL request form is available on our website: https://nvrl.ucd.ie

6.7 Outbreak Investigations

The NVRL is very willing to work with users in the investigation of single- or multi-sight outbreaks. If the pathogen in question is notifiable, we recommend that you first inform Public Health and then a member of our clinical team (01 716 4401) to establish a protocol for the priority testing of samples and the communication of results.

In circumstances where a large number of samples are expected, it can be useful to develop a unique test request form & test request code so the samples are easily identifiable on arrival at the NVRL.

6.8 Supplementary Testing

Please note that on occasion, where indicated, a member of the NVRL clinical team may perform extra tests on a sample: this procedure typically occurs following review of the clinical details, and is intended to clarify the significance of initial test results.

Additional Turnaround Time: Please note that additional time may be required for confirmation/supplementary testing of initially reactive samples. Furthermore, samples referred to other reference laboratories may extend the test turnaround time.

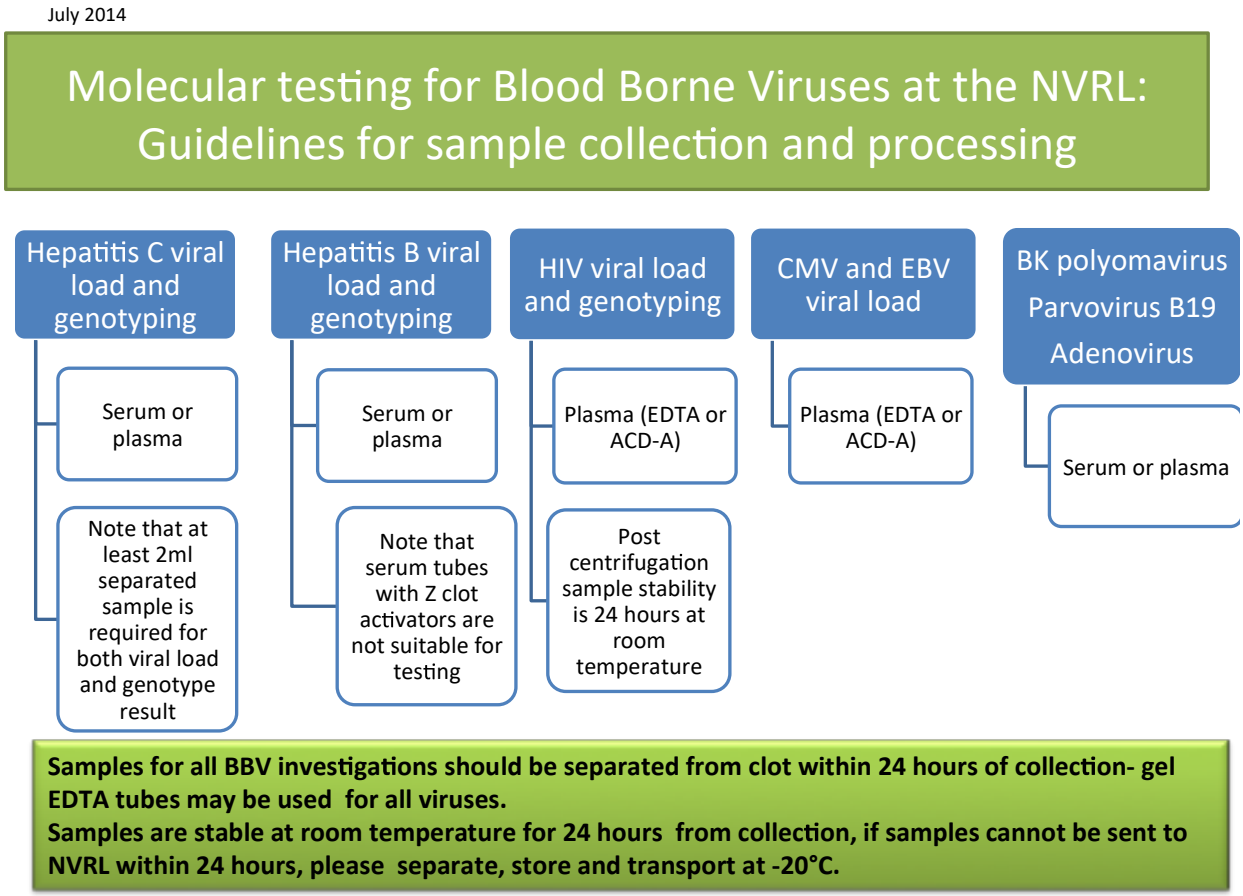
6.9 Same Day Results

The NVRL is committed to providing a clinically relevant service, and appreciates that laboratory results frequently inform further investigations & patient management. As such, it is important for users to note that depending on the test being performed (Molecular testing typically requires 6-8 hours) same day results are not always possible. Therefore, therapeutic (or infection control) decisions for unwell patients should not be deferred pending laboratory results.

However, as a general rule – and providing the NVRL is informed that the sample is on route (see section 2.11 above, Urgent Laboratory Investigation) – the majority of tests can generate a same day result ***if*** the sample is received in the laboratory ***no later than*** 10:30hrs

The minimum turnaround time for a single test is calculated from date of receipt of specimen to date of issue of report in working days. Any sample arriving after 12.00 hrs will be regarded as arriving the next working day. Please note that additional days are required for confirmation of reactive samples. Samples referred to other reference laboratories will further extend the test turnaround time. Please refer to the Virus Testing Index at https://nvrl.ucd.ie

6.10 Blood Borne Virus (BBV) Screening



Whole blood samples received for proviral load testing are sent to Public Health England unspun and must tested within 3 days of the sample date. Samples cannot be sent over the weekend. The following workflow time-frame is suggested in order to meet these requirements:

1. **Blood is drawn on MONDAY and sent immediately to the NVRL**
2. **Whole blood sample should be received at NVRL by TUESDAY**
3. **Whole blood will be referred to UK on WEDNESDAY**
4. **Whole blood sample should be received by PHE by THURSDAY.**

NVRL identified the handling of Blood Tubes for Molecular Investigation as a Risk, and produced a bulletin in December 2022 (CN-22-22). A copy of this is available by emailing nvrl.qa@healthmail.ie

7. REFERENCE RANGES

Based upon the nature of the investigations, either direct viral detection or the immune response to viral infection, diagnostic virology tests do not conform with a reference range. Where clinically relevant titres exist, in the case of post vaccine response, the NVRL follows guidance as set out in the following:

https://www.hse.ie/eng/about/who/cspd/ncps/pathology/resources/national%20laboratory%20handbook.html

https://www.rcpi.ie/healthcare-leadership/niac/immunisation-guidelines-for-ireland

https://www.hse.ie/eng/about/who/cspd/ncps/pathology/resources/suspected-viral-hepatitis1111.pdf

Any further queries regarding reference ranges can be sent by email to nvrl.qa@healthmail.ie

8. NVRL PATHOGEN INDEX: ALPHABETICAL

For information regarding the following: testing strategies, sample types tested, frequency of testing and Turnaround Times (TATs) please go to our website https://nvrl.ucd.ie/info and look for our Virus Testing Index (LF UM 001r).

Turnaround times are calculated on working days, and in most cases, do not include confirmatory tests. Refer to Section 6.8

Tests done 'ON REQUEST' may have turnaround times shorter than those listed.

(Where samples are referred to the Rare and Imported Pathogens Lab, in the UK, this refers to RIPL, PHE Porton Down.)

Paragraphs detailing Viruses not tested for at the NVRL are headed in **RED**

9. VIRAL TAXONOMY

- There are constant reviews of Viral Taxonomy made by the **International Committee on Taxonomy of Viruses ICTV**, see link: https://talk.ictvonline.org/taxonomy/

- The article, "Why virus taxonomy is important", published in *Microbiology Today*, 13 February 2018, by Stuart Siddell, ICTV Vice President, discusses the importance of virus taxonomy and its role in helping to define the evolutionary relationships between viruses and understand the consequences of virus diversity.

- Respiratory syncytial virus (RSV) has been recently re-named as Human orthopnuemovirus, and parvovirus B19 has been re-named as Primate erythroparvovirus 1. These names are both included in the Virus Testing Index, LF UM 001r to be found on the NVRL website.

Adenovirus

Introduction: Adenoviruses (ADV) are double-stranded DNA viruses for which humans are the natural host. The ADV genus is divided into a number of different species (A-G) and serotypes (1-52).

Clinically: Adenoviruses are typically associated with respiratory tract infections, gastroenteritis (Adeno group F), and conjunctivitis, although they can also cause haemorrhagic cystitis and systemic disease in immunocompromised individuals. The viruses are primarily transmitted by direct contact, aerosol, and faeco-orally; in addition, ocular instruments may become contaminated leading to outbreaks of ADV conjunctivitis.

Diagnosis: Adenoviruses can be detected in respiratory secretions (NPA, BAL), blood (plasma/serum), stool samples, conjunctiva, throat swab, cerebrospinal fluid (CSF) and urine, depending on the clinical syndrome.

NVRL Tests: ADV infections are confirmed by molecular diagnosis (PCR) NVRL has 4 different PCRs for Adenovirus diagnosis.
There is an adenovirus component to the Respiratory Screen; Adenovirus species F (ADV 41) is one component of the Gastroenteritis Screen.
Swabs and Post Mortem samples can also be tested. EDTA Plasma, serum and CSF samples are all tested using a 4th assay.

Pathogen-Specific Notes:
1. The inclusion of clinical details is of utmost importance in assisting selection of the appropriate testing strategy, particularly in suspected oncology or haematology patients.

Arbovirus

Introduction: Arbovirus is a word (derived from ***ar***thropod-***bo***rne) applied to a diverse group of viruses from different families that are typically transmitted by mosquito bite. The NVRL possesses two distinct Arbovirus panels that are used based on the patient's travel history. There is a standard Euroimmun Flavivirus IFA screen*. Information on the individual viruses is presented on their own page.

Clinically: While the majority of infections caused by these viruses are asymptomatic, or cause a self-limiting febrile illness, as the names suggest they all have the capacity to cause severe encephalitis in a percentage of patients.

Diagnosis: While the virus itself can be detected (in blood or occasionally CSF) by PCR early in the course of infection, serology (specifically IgM) is the mainstay for diagnosis, as the viraemic phase of the illness is typically short-lived.

NVRL Tests: Arbovirus infections can be confirmed by detecting the presence of IgM antibody in serum or clotted blood. Confirmatory testing for arboviruses is performed (as necessary) by Public Health England Rare & Imported Pathogens Unit in the UK.

***Screen:** West Nile Virus, Japanese Encephalitis Virus, Yellow Fever, Dengue Virus, Tick Borne Encephalitis Virus.

Please note an accurate travel history is particularly important to ensure the appropriate Arbovirus screen is performed.

Please also notify the laboratory if the patient has received Yellow Fever Virus (YFV) vaccine, as YFV antibodies may cross react with other viruses in the Flavivirus screen, yielding false positive results.

Astrovirus

Introduction: Astroviruses are small single-stranded RNA viruses, of which there are 8 genotypes that cause infection in humans. Although also found in many different species of animal worldwide, the viruses are species-specific so humans are the natural reservoir for the viruses that cause human disease.

Clinically: Astrovirus infection may be asymptomatic, but typically causes an acute diarrhoeal illness that primarily affects infants and the elderly. Indeed, 75% of children are astrovirus antibody positive by the age of ten years. Astrovirus outbreaks may also occur as a result of person-to-person transmission or from a common foodborne source. The virus is transmitted faeco-orally.

Diagnosis: Astroviruses can be detected in stool samples from symptomatic patients.

NVRL Tests: Astrovirus PCR forms one component of the MGAS group test for the diagnosis of causative agents of gastrointestinal infection of viral aetiology.
Other viruses in this panel include Adenovirus group F (ADV 41); Rotavirus wild-type and vaccine derived strain; Sapovirus; Norovirus Genogroup 1 and Genogroup 2.

BK Polyomavirus

Introduction: BK Polyomavirus (BKV) is a double-stranded DNA virus, of which there are four subtypes (I-IV). The virus is named after the initials of the patient from whom it was first isolated in 1971.

Clinically: BKV is distributed worldwide with humans as natural hosts. Primary infection with BKV typically occurs in childhood and does not cause significant illness in otherwise healthy individuals. Indeed, as a consequence of the asymptomatic nature of primary infection, the route of transmission has not been definitively established. In immunocompromised patients however, BKV is the cause of significant morbidity, causing Polyomavirus associated nephropathy (PVAN or BKVAN) in solid organ transplant recipients, and haemorrhagic cystitis in haematopoietic stem cell transplant recipients.

Diagnosis: BKV can be detected in urine (viruria) and blood (viraemia) from both symptomatic and asymptomatic patients.

NVRL Tests: BK viruria can be confirmed using EM or PCR, and viraemia can be confirmed by PCR. Please note that BKV viral load in blood may be performed on plasma or serum: it is important if monitoring a patient that the same sample type is used on each occasion to ensure sequential viral load results are comparable.

Bocavirus

Introduction: Human bocaviruses (HBoV) are (recently described) single stranded DNA members of the Parvovirus family. The name derives from the similarity of the virus to ***bo***vine & ***ca***nine parvoviruses. There are currently 4 proposed HBoV genotypes (1-4).

Clinically: The true disease burden of HBoV remains to be clarified, as it has been detected in respiratory samples taken from people with respiratory illness, and also in stool samples taken from individuals with diarrhoea. The majority of the evidence supports its role in respiratory tract infection, although whether it acts in isolation or in conjunction with other viral pathogens remains unclear.

Diagnosis: HBoV can be detected in respiratory secretions of infected individuals, although asymptomatic shedding (HBoV DNA in healthy controls) has been reported.

NVRL Tests: HBoV testing is currently performed by PCR as one component of the Respiratory testing screen

Borrelia burgdorferi (Lyme Disease)

Introduction: Lyme disease is a tick-borne infection caused by the spirochete *Borrelia burgdorferi*. The diagnosis of Lyme Disease should be based primarily on the clinical presentation and an assessment of tick-exposure risk.

Clinically: *Borrelia burgdorferi* infection may be asymptomatic. Symptomatic infection (Lyme disease) is potentially multisytemic and may have dermatological, neurological, musculoskeletal and/or cardiac manifestations. For further information please refer to best practice guidelines issued by the British Infection Association (available on the NVRL website).

Diagnosis: Serological testing for antibodies to *B. burgdorferi* is the mainstay of diagnostic testing. If acute infection is suspected please refer to the BIA guidelines and contact the NVRL Clinical Team to discuss if required. In relation to suspected Neuroborreliosis – please see below. **If investigation of CSF for *B. burgdorferi* is required , CSF samples without a contemporaneous serum will not be processed. These samples should be sent directly from the source laboratory to RIPL, Porton Down (see contact information below).**

NVRL Tests: A two-stage approach for the serological diagnosis of *B burgdorferi* infection is currently applied by the NVRL in line with best practice guidelines. A sensitive enzyme immunoassay (Liaison *B burgdorferi* IgG enzyme immunoassay) is used as a first (screening) step. Samples yielding reactive screening test results are referred to the Lyme Borreliosis Unit (Lyme RU) Public Health England (PHE) Porton for further investigation by second-stage immunoblot (Western blot) tests. Please note samples will not be referred to the LRU without clinical details as this information is essential to aid in result interpretation. *B burgdorferi* IgM testing is not performed in the NVRL.

Molecular Testing: *B burgdorferi* PCR is not performed by the NVRL. Detection of *Borrelia* DNA by polymerase chain reaction (PCR) is diagnostically useful in certain well-defined circumstances, e.g. tissue biopsy and investigation of synovial fluid. However it is of limited value in the investigation of CSF (Borrelia DNA is detectable in only 10-30% of patients with proven acute neuroborreliosis), and is not recommended for the testing of urine or blood samples.
If PCR investigation is required please contact the NVRL Clinical Team at 01-7164418 to discuss As of 4th January 2022, paired sera and CSF samples for the diagnosis of neuroborreliosis will not be referred from NVRL. These samples should be sent to RIPL, Porton Down, by the source laboratory. Please see nvrl.ucd.ie for the relevant customer notification. (CN-21-21)

Refer to Rare and Imported Pathogens (RIPL) User Manual, page 17: volume of CSF required for diagnosis of Lyme neuroborreliosis = 600µl.

Contact information for RIPL, Porton Down can be found below.
RIPL,
Operations
National Infection Services
Manor Farm Road, Porton Down

Salisbury, SP4 0JG, UK
+44(0)1980 612348

https://www.gov.uk/government/publications/rare-and-imported-pathogens-laboratory-ripl-user-manual

Bunyamwera Virus

Introduction: Bunyamwera virus (named for the town in Uganda in which it was first discovered) is a single-stranded RNA virus whose name has been taken for the Bunyaviridae family of viruses. The virus is widely distributed in Africa, and transmitted by mosquitoes, although the natural reservoir is undetermined. The Bunyaviridae family is divided into five genera, including four that infect humans: Bunyavirus; Phlebovirus; Nairovirus; & Hantavirus. Of the better-known human pathogens, Crimean Congo Haemorrhagic Fever Virus is a member of the Nairovirus genus, and Toscana virus is a member of the Phleboviruses.

Clinically: As the original Bunyavirus, Bunyamwera virus is more significant for its name than its clinical syndrome. Human infection is rare, although when it does occur it can be significant, typically leading to fever, rash, and arthralgia. Encephalitis and temporary loss of vision can also occur.

Diagnosis: Bunyamwera virus can be detected (PCR) in the blood of infected individuals, although diagnosis is typically made serologically. **This testing requires contact with NVRL before submission of the sample.**

NVRL Tests: Bunyavirus infection can be confirmed serologically: these samples are referred to the Rare and Imported Pathogens Unit in the UK.

Calicivirus

Introduction: Caliciviruses are small single-stranded RNA viruses that are more commonly referred to by their species names, Norovirus & Sapovirus. They are also known by the term SRSV (small round structured viruses) on account of their appearance under the electron microscope. The virus family was named for the cup-like indentations (Latin: calyx, cup) visible on the virus surface on EM. Both Norovirus and Sapovirus species contain 5 genogroups, with multiple genotypes within each. In a similar fashion to influenza (albeit less frequently), the predominant circulating strain of Norovirus varies over time. Caliciviruses are distributed globally and transmitted faeco-orally and by aerosol.

Clinically: Caliciviruses are the second most important cause of viral gastroenteritis (after Rotavirus). Although asymptomatic infection (30%) and persistent infection (in immunocompromised) can occur, the classic presentation is of sudden onset vomiting and/or diarrhoea that typically last 12-48 hours.

Diagnosis: Caliciviruses can be detected in stool and vomitus of infected individuals, although stool is the preferred sample type.

NVRL Tests: Suspected Norovirus infection is confirmed by PCR, Norovirus genogrouping is also routinely performed (by PCR), and distinguishes between genogroup I and II.
Norovirus PCR forms part of the MGAS Panel for the diagnosis of causative agents of gastrointestinal infection of viral aetiology.
Other viruses in this panel include Adenovirus group F; Astrovirus; Rotavirus wild-type and vaccine derived strain; Sapovirus.

Chikungunya Virus

Introduction: Chikungunya virus (CHIKV) is major cause of epidemic arthralgic illness in Africa and Asia. The virus is a single-stranded RNA alphavirus of the Togaviridae family. The name is derived from the Swahili for 'that which bends up', a reflection of the severe joint pain that the infection causes. CHIKV is a mosquito-transmitted virus that has traditionally been distributed throughout Africa and Southeast Asia, but more recently has been introduced into Southern Europe and is endemic in the Caribbean and South America. Although the natural hosts of the virus in Africa are non-human primates, it would appear that human hosts are sufficient to sustain the virus in Asia.

Clinically: Fever, rash, and joint pains constitute the classic triad of CHIKV infection, but haemorrhage and encephalitis may also occur.

Diagnosis: Although CHIKV infection can be diagnosed clinically in most cases (if there is an appropriate travel history), the virus can also be detected in the blood of infected individuals.

NVRL Tests: CHIKV infection can be confirmed serologically (from the 2nd week of illness) by immunofluorescence or by PCR if the patient present early in the course of the illness

Chlamydia trachomatis

Introduction: *Chlamydia trachomatis* (CT) is a small obligate intracellular gram negative bacterium. There are fifteen different serovars causing distinctive clinical syndromes. Natural infection confers only short-lived protection against reinfection. CT in the developed world is well established as a leading sexually transmitted infection (STI).

Clinically: The different serovars of CT are associated with different clinical syndromes. Serovars D-K: sexually transmitted CT infection may be asymptomatic or present as epididymitis, urethritis, salpingitis or cervicitis. Infection in females can lead to pelvic inflammatory disease and infertility. Serovars D-K may also cause inclusion conjunctivitis & pneumonia in the neonate. Serovars L1-3 causes lymphogranuloma venerum (LGV), an STI that initially presents as a small ulcer or papule on genital mucosa or nearby skin that heals rapidly. Secondary symptoms then develop and include lymphadenopathy, abscesses, fever, headache, myalgia, proctitis, and occasionally meningitis. Serovars A-C cause Trachoma, a chronic follicular kerato-conjunctivitis, corneal scarring & the commonest cause of preventable blindness in the developing world.

Diagnosis: Nucleic Acid Amplification tests (NAATs) are the preferred investigation for diagnosis of CT infection. Dual testing for CT & *Neisseria gonorrhoeae* is routine.

NVRL Test: Combined CT and *N gonorrhoeae* (NG) testing is performed in the NVRL. Specimen collection advice is included in the APTIMA kits provided by the NVRL. Three types of specimen collection device (SCD) are available: vaginal swab; Unisex/endocervical swab; and urine. Following specimen collection, the SCD are stable at room temperature for 60 days.

Pathogen-Specific Notes:

1: Only specimens collected in APTIMA collection devices can be tested in the NVRL.

2: Please refer to section 5.5 Specimen Collection for information related to the APTIMA collection devices, Please pay particular attention to the sample level required in the SCD.

3: Clinicians are advised to refer to existing guidelines in relation to the need for ***test of cure*** following treatment. NAATs may detect residual Chlamydia nucleic acid even after successful treatment of the organism for four to six weeks after treatment.

4: NAAT positive ***rectal swabs*** for CT are referred to the Bacteriology Reference Laboratory, (STBRU) in PHE Colindale for further testing to identify LGV serovars

Chlamydophila pneumoniae/Chlamydia pneumoniae

Introduction: *Chlamydophila pneumoniae / Chlamydia pneumoniae* (*C. pneumoniae*) an atypical bacterial pneumonia characterized by a relatively long incubation period, 3 to 4 weeks with symptoms beginning 3 to 4 weeks following exposure. The bacterium is most commonly associated with community-acquired pneumonia. He majority of individuals are first infected in childhood; however, reinfection is common in older adults.

Clinically: Clinical presentation is non-specific and may include laryngitis, pharyngitis, coryza, malaise, fever, cough, and headache. Specifically, *C. pneumoniae* may be differentiated from *Mycoplasma pneumoniae*, by the presence of laryngitis, which the majority of symptomatic patients develop. The majority of infections are asymptomatic or mild, although severe complications including severe pneumonia, exacerbation of asthma, encephalitis and myocarditis have been reported. *C. pneumoniae* respiratory infection may continue for extended periods, with a persistence of symptoms for several weeks or months despite appropriate treatment.

Diagnosis: *C. pneumoniae* infection may be diagnosed by culture, serological and molecular-based laboratory techniques.

NVRL Tests: *C. pneumoniae* infection can be confirmed by the presence of *C. pneumoniae* nucleic acid in bronchoalveolar lavage (BAL), sputa and other lower respiratory tract patient specimens.

Coronavirus

Introduction: Coronaviruses are single-stranded RNA viruses that can be divided into three groups (1-3). Until 2003, there were two main human coronaviruses (HCoV 229E & HCoV OC43) that were responsible for about 25% of common cold cases, but did not cause significant morbidity or mortality. In 2003 however, a novel coronavirus was discovered – the severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) – that was substantially more pathogenic with a mortality rate of about 10%. The arrival of SARS-CoV led to renewed interest in coronaviruses, and subsequently two more novel viruses were discovered – HKU1 & NL63 – but these were not very pathogenic. More recently, however, Middle Eastern Respiratory Syndrome (MERS) associated coronavirus (MERS-CoV) has emerged in Saudi Arabia and Qatar. At the time of writing, the mortality rate for MERS-CoV is >40%.
The emergence in Quarter 1, 2020 of infections caused by a Novel Coronavirus, subsequently named as SARS-CoV-2 with the resulting disease termed COVID-19, led to a global pandemic. Please see page 104 for details.

Clinically: As mentioned above, coronavirus infections range from the common cold to SARS & MERS. In addition, a significant number of non-SARS non-MERS coronavirus infections are asymptomatic.

Diagnosis: Coronaviruses can be detected in respiratory specimens from infected individuals.

NVRL Tests: Suspected coronavirus infection (HCoV 229E, HCoV OC43 and HKU1) can be confirmed by PCR. These viruses are detected as components of the NVRL Viral respiratory Screen,(current code LRSC)

Pathogen Specific Notes: If MERS-CoV infection is suspected, public health should be notified prior to requesting laboratory testing. Please refer to the Health Protection Surveillance Centre website for the most up to date guidance www.hpsc.ie

Crimean Congo Haemorrhagic Fever Virus

Introduction: Crimean Congo Haemorrhagic Fever Virus (CCHF) is a single-stranded RNA virus, from the Nairovirus genus of the Bunyaviridae family. The virus is widely distributed over Eastern Europe, Asia, and Africa (hence, the name) and is transmitted by the Ixodid tick. The natural reservoir for the virus includes the small mammals (hares) and large herbivores (cows, sheep, goats) on which ticks feed.

Clinically: CCHFV infection typically manifests as a severe febrile illness with rash which progresses to severe haemorrhage and multiorgan failure. The case fatality rate is about 30%, with death occurring in the second week of illness.

Diagnosis: The virus can be detected in blood early in the course of illness, with antibody detectable after the first week.

NVRL Tests: Suspected CCHFV infection can be confirmed by PCR and/or serology. Samples are initially screened (in CL3 laboratory) at the NVRL, with supplementary and confirmatory testing performed in Rare & Imported Pathogens Laboratory in the UK.

Viral Haemorrhagic fever (VHF) testing is only done by prior arrangement with clinical team and as per guidelines at:
www.hpsc.ie/hpsc/A-Z/Vectorborne/ViralHaemorrhagicFever/Guidance/
The NVRL must be notified before dispatch from hospital of any suspect VHF specimens. Please contact the laboratory on 01-7161321/7164440.

Cytomegalovirus

Introduction: Cytomegalovirus (CMV) is a double-stranded DNA virus that is a member of the Herpesviridae family. Although there are three CMV genotypes, there is no established disease association at this time. Humans are the natural reservoir for CMV and the virus is distributed worldwide. CMV can be transmitted in saliva, through sexual contact, or vertically from mother to child.

Clinically: In otherwise well individuals, CMV infection may be asymptomatic or cause a glandular-fever type illness. If infection occurs during pregnancy, congenital infection can occur, the most common manifestation of which is sensorineural hearing loss. CMV infection in immunocompromised patient cohorts may cause retinitis, pneumonitis, hepatitis, colitis, and bone marrow suppression.

Diagnosis: CMV can be detected in the urine of most infected individuals, the urine, saliva (& sometimes blood) of congenitally infected infants, and the blood of immunocompromised individuals.

NVRL Tests: Primary CMV infection is typically confirmed serologically in immunocompetent individuals. Congenital infection is confirmed via PCR to detect CMV in urine or saliva within the first 21 days of life. CMV infection in immunocompromised individuals may be confirmed either serologically or by PCR. Please note that end-organ CMV disease such as colitis requires a histological diagnosis to be definitive.
CMV tests can be carried out on Dried Blood Spots (DBS) from infants. These are available from and managed by CHI@Temple St. Results from these samples will be sent to the requesting source, not to CHI@Temple St.

Pathogen Specific Notes:

1: CMV infection of the central nervous system (CNS) is not common in otherwise well individuals: CSF testing for CMV DNA should only be performed in parallel with serology and DNA testing in blood (EDTA sample)

Dengue Virus

Introduction: Dengue (DENV) is a single-stranded RNA member of the Flavivirus family, of which there are four serotypes. DENV is considered the most important arboviral cause of death and disease in humans, with more than 100 million infections/year. Between 250000 and 500000 will develop dengue haemorrhagic fever, which carries a 5% case fatality rate. The virus is transmitted by mosquito and is endemic in the tropics, and can survive wherever the mosquito host is present.

Clinically: Although the majority of dengue virus infections are asymptomatic, infection can result in the classic biphasic febrile illness (dengue fever) that may progress to haemorrhage (dengue haemorrhagic fever, DHF), and shock. DHF is more common in secondary infection and in the young.

Diagnosis: Dengue virus RNA may be detected in the blood of infected individuals early in the course of infection, but IgM is the more commonly seen marker of recent infection.

NVRL Tests: Dengue virus serology and RT PCR is performed in the NVRL, with confirmatory testing performed in the Rare & Imported Pathogens Laboratory in the UK.

Pathogen-Specific Notes:

1: When suspecting imported viral infection, please provide a comprehensive & detailed travel history to ensure appropriate and timely testing. If you are uncertain of the potential pathogens, a member of the NVRL clinical team would be happy to discuss. (Phone: 01-7164401)

Eastern Equine Encephalitis Virus

Introduction: Eastern equine encephalitis virus (EEEV) is a single-stranded RNA alphavirus from the Togaviridae family, and is the most severe arthropod-borne viral cause of encephalitis, with a mortality of 35%. The virus is distributed throughout the US and South America, with the Eastern in the name referring to the Eastern US. Birds are the natural hosts for EEEV, and mosquitoes transmit the virus: as such, humans and horses are in reality 'dead-end' hosts.

Clinically: The majority (90-95%) of human infections are asymptomatic. However, when encephalitis does result, it carries a mortality of 35% and the majority of those who survive will suffer some residual disability.

Diagnosis: Virus can be detected by PCR in blood if the patient presents early in the course of infection, and in post-mortem brain tissue. However, IgM is more commonly used as a marker of recent infection.

NVRL Tests: EEEV is not tested at the NVRL. Specimens are therefore referred to the Rare & Imported Pathogens Laboratory in the UK for testing.

Pathogen-Specific Notes:

1: When suspecting imported viral infection, please provide a comprehensive & detailed travel history to ensure appropriate and timely testing. If you are uncertain of the potential pathogens, a member of the NVRL clinical team would be happy to discuss.

Enterovirus (including EV-A71 and EV-D68)

Introduction: Enteroviruses (EV) are small single-stranded RNA viruses that belong to the Picornaviridae family. Presently, EV can be divided into 3 main groups (A-C) with multiple subgroups in Groups B & C: as such, there are more than 100 circulating EV subtypes. Humans are the natural host for EV and the viruses are distributed globally. EV transmission occurs either faeco-orally or via respiratory secretions, with infections typically peaking in the summer months. Of note, Coxsackie virus and Echovirus are EV subtypes.

Clinically: EV cause a wide range of syndromes including: hand, foot and mouth disease (HFMD); aseptic meningitis; acute haemorrhagic conjunctivitis; myocarditis; neonatal sepsis; skin rashes; and respiratory illness. Despite the name (the GIT is the site of replication), EV do not typically cause diarrhoea. In recent years, Enterovirus A71 has emerged as a significant cause of brainstem encephalitis (leading to severe pulmonary oedema, cardiac dysfunction, and death) and acute flaccid paralysis, especially in Southeast Asia and the Far East. Similarly, Enterovirus D68 causes mild to severe respiratory illness, sometimes even requiring ventilator support, but has also been associated with cases and clusters of polio-like neurological symptoms, including acute flaccid paralysis (AFP).

Diagnosis: EV can be detected in stool samples, respiratory secretions, CSF, blood, and vesicular fluid (HFMD).

NVRL Tests: NVRL primarily diagnoses EV infection by molecular testing (PCR). A faecal sample is the specimen of choice for enterovirus culture. Molecular based characterisation (typing) is carried out on selected samples with a positive PCR result on the advice of NVRL Clinical staff. Serology testing for EV is ***not*** available.

Pathogen Specific Notes:

1: Enterovirus RNA testing in the NVRL screens for clinically relevant Enterovirus types currently circulating: results are reported as Enterovirus Detected or Not Detected. Typing of specific EVs is possible following molecular testing and/or culture of the virus.

Epstein Barr Virus

Introduction: Epstein Barr Virus (EBV) is a large double-stranded DNA virus from the Herpesviridae family. Although there are two strains (1/2 or A/B), these do not yet have any significant clinically distinguishing features as yet. EBV-1 is the more prevalent virus in the western world. EBV is distributed globally and humans are the natural host. Like other members of the herpesvirus family, EBV remains latent for life in the infected individual. Reactivation can asymptomatic viral shedding can occur. EBV is transmitted in saliva.

Clinically: EBV is the primary causative agent of glandular fever, (also known as infectious mononucleosis, or kissers' disease (in the USA)). In the majority of otherwise well individuals, primary infection is asymptomatic or causes a self-limiting infection. However, some individuals do experience a more profound post-infectious fatigue that can last for up to 6 months. EBV also has oncogenic potential, being primarily associated with B cell lymphomas & nasopharyngeal carcinoma, although it may also play a role in some T cell lymphomas. In addition, in the transplant setting, EBV infection can lead to post transplant lymphoproliferative disorder (PTLD).

Diagnosis: Primary EBV infection is typically a serological diagnosis, with reactivation diagnosed or monitored by the detection of EBV DNA in blood. EBV DNA can also be detected in salivary fluid, however saliva is not tested at NVRL

NVRL Tests: Suspected primary infection can be confirmed by the presence of IgM and IgG directed at the viral capsid antigen (VCA). Antibody to the EBV nuclear antigen (EBNA) typically takes 8-12 weeks to appear and remains detectable for life. EBV DNA (viral load) testing is not required in primary infection, but is used for the monitoring of patients at risk for PTLD. EBV DNA testing requires an EDTA sample.

Pathogen Specific Notes:

1: EBV infection of the central nervous system (CNS) is not common in otherwise well (immunocompetent) individuals: CSF testing for EBV DNA should only be performed in parallel with serology and DNA testing in blood (EDTA sample)

EBV PCR can be performed on plasma samples or whole EDTA blood samples, which are not spun and stored at +4oC- It is important that forms are clearly marked with the sample type to ensure appropriate processing in specimen reception.

Filovirus

Introduction: Filoviruses are single-stranded RNA viruses that cause haemorrhagic fever. There are two main genera named after the most famous virus members: Ebola & Marburg. The Ebola-like genus contains 4 subtypes. Fruit bats are the natural reservoir for filoviruses and humans typically become infected following exposure to ill primates. The viruses are distributed across the belt of Africa, and person-to-person spread occurs through unprotected direct contact with blood or infected secretions from an infected individual. The incubation period for filoviruses ranges from 3-12 days.

Clinically: The classic Ebola virus presentation is febrile illness with headache, rash, abdominal pain, and diarrhoea progressing to haemorrhage and death secondary to hypovolaemia. Asymptomatic infection has been reported with Ebola-Reston virus.

Diagnosis: Viral RNA can be detected in blood and secretions during the course of the illness.

NVRL Tests: Initial testing for filoviruses in the NVRL is carried out in accordance with national guidelines*. Supplementary testing is performed in the Rare and Imported Pathogens Laboratory in the UK.

Viral Haemorrhagic fever (VHF) testing is only done by prior arrangement with clinical team and as per guidelines at:
*www.hpsc.ie/hpsc/A-Z/Vectorborne/ViralHaemorrhagicFever/Guidance/
The NVRL must be notified before dispatch from hospital of any suspect VHF specimens. Please contact the laboratory on 01-7161321/7164401

GB Virus C

Introduction: GB Virus C is a single-stranded RNA flavivirus that was isolated from a surgeon (whose initials were GB) with hepatitis in 1966. Subsequent seroprevalence studies revealed that the virus was distributed globally, albeit with an increased prevalence in Africans. While humans are considered the natural host, the virus has also been detected in primates. Five genotypes of the virus (1-5) have been recorded to date.

Clinically: Despite its initial discovery in an individual with hepatitis, there is no evidence that GB virus C (GBV-C) is hepatotropic, and there is as yet no definitive disease association. Of interest however is the fact that, in addition to other cells in peripheral blood, GBV-C replicates in CD4+ T cells, and in so doing appears to reduce the capacity for HIV to enter the cell. This may be due to an impact on CCR5 expression.

Diagnosis: GBV-C virus RNA can be detected (by PCR) in the blood of individuals early in the course of infection.

NVRL Tests: There is presently no diagnostic test routinely available for GBV-C*.

*Following initial discovery of the new virus particle, serum from the surgeon was injected into tamarind monkeys, who subsequently developed hepatitis. Some thirty years, later, acute phase serum from these experiments was analysed and two viruses were identified, termed GBV-A & GBV-B. However, neither of these was detected in the serum from the surgeon, so the original isolate was termed GBV-C.

Hantavirus

Introduction: Hantavirus belongs to the Bunyaviridae family and as such is a single-stranded RNA virus. The name derives from the Hantaan River in Korea where the virus was first isolated, although the virus is distributed globally. There are four antigenic Hantavirus types: Hantaan; Puumala; Sin Nombre; and Thottapalayam. These have also been named for the place in which they were first detected. Rodents (mice, rats, voles) are the natural hosts and humans are infected by dust contaminated with rodent excreta. Human to human transmission is rare, although it has been reported.

Clinically: Hantavirus infection causes two main syndromes, depending on the virus variant involved. Sin Nombre virus, which predominates in the US causes Hantavirus (Cardio) Pulmonary Syndrome (HPS) and Hantaan & Puumala (Asia & Europe) cause a Haemorrhagic Fever with Renal (Failure) Syndrome (HFRS). Both syndromes can be fatal, but early diagnosis and intensive care support can reduce mortality.

Diagnosis: Virus can be detected (by PCR) in infected individuals early in the course of illness, but IgM antibody is typically present by the time of hospital admission.

NVRL Tests: Initial Hantavirus serology is performed in the NVRL, with supplementary testing performed in the Rare and Imported Pathogens Laboratory in the UK.

Pathogen-Specific Notes:

1: When suspecting imported viral infection, please provide a comprehensive & detailed travel history to ensure appropriate and timely testing. If you are uncertain of the potential pathogens, a member of the NVRL clinical team would be happy to discuss.

Heartland Virus

Introduction: Heartland Virus (HV) is a single-stranded RNA virus from the Phlebovirus genus of the Bunyaviridae family that was discovered in 2009 from 2 Northwest Missouri farmers in the US. Based on the patients' exposures, the virus was believed to be transmitted by ticks and has subsequently been detected in the Lone Star tick (*Amblyomma americanum*). To date, only a handful of additional cases (in Missouri & Tennessee) have been reported with one death in a patient with multiple underlying comorbidities.

Clinically: Based on this small number of infections, Heartland virus presents as a febrile illness with leucopaenia and thrombocytopaenia. Fatigue, myalgia, arthralgia, and headache have also been reported. Seroprevalence studies will be required to define the true spectrum of disease.

Diagnosis: HV RNA can be detected in the blood and tissues of infection individuals. Acute and convalescent serology (plaque reduction neutralisation assays) can also confirm infection.

NVRL Tests: There is currently no test available for Heartland Virus in the NVRL, nor is there a commercial assay available. In an individual with the appropriate clinical presentation and travel history, assuming other infectious pathogens have been excluded, the NVRL can liaise with the CDC to arrange HV testing.

Hendra Virus

Introduction: Hendra virus is a single-stranded RNA virus that belongs to the Henipavirus genus of the Paramyxoviridae. The virus is named for the suburb of Brisbane in Australia where it was first discovered, and continues to cause sporadic deaths in horses and trainers. The natural host for the virus is the pteropid bat (flying fox), but humans appear to be infected from the urine or saliva of infected horses. No human to human transmission has been reported.

Clinically: Hendra virus infection causes an influenza-like illness, with headache and meningitis, but can progress to multiorgan dysfunction. In addition, although human cases are rare, it also has the capacity to cause a relapsing encephalitis, which has been compared to the sub-acute sclerosing pan-encephalitis (SSPE) seen with Measles virus infection.

Diagnosis: Hendra virus can be detected by molecular (PCR) and serological methods, but it is a biosafety level 4 pathogen.

NVRL Tests: Testing for Hendra virus is not presently available at the NVRL. Samples from patients with the appropriate travel history and presentation are referred to the Rare & Imported Pathogens Laboratory in the UK.

Hepatitis A Virus

Introduction: Hepatitis A virus (HAV) is a single-stranded RNA virus from the hepatovirus genus of the Picornaviridae. Although there are 6 genotypes, at present there is no significant clinical association for different genotypes. Humans are the natural host for HAV and the virus is endemic in all parts of the world. Transmission from person to person occurs via the faecal-oral route: as such, the virus can also lead to foodborne outbreaks and is classically associated with shellfish.

Clinically: Hepatitis A virus is an acute disease of the liver typically causes an acute hepatitis, presenting with anorexia, fever, nausea, abdominal pain, and jaundice. Some people experience a fairly mild illness and recover within a couple of weeks. Other people develop more severe symptoms and may take months to fully recover. Older people are likely to have more severe symptoms However, the infection is often asymptomatic in children. HAV does not cause chronic hepatitis. Hepatitis A became notifiable in 1981In developed countries, hepatitis A is most commonly seen among travellers to endemic countries, household or sexual contacts of known cases, people who inject drugs (PWID) and men who have sex with men (MSM)

Diagnosis: HAV can be detected in the stool and serum of infected individuals (by PCR).

NVRL Tests: HAV infection can be confirmed by documenting the presence of specific IgM in the serum of infected individuals. HAV RNA can also be detected in stool, EDTA bloods & serum, and genotyping can be performed if a suspected outbreak is being investigated.
Acute hepatitis screening includes testing for HAV IgM.
HAV PCR is performed weekly

Pathogen Specific Notes:

1: Hepatitis A is a vaccine preventable infection: patients with chronic Hepatitis B infection, chronic Hepatitis C infection, and other forms of chronic liver disease should be routinely considered for HAV vaccination

2: Post Vaccination testing for anti-HAV IgG is not indicated

Hepatitis B Virus

Introduction: Hepatitis B virus (HBV) is a double-stranded DNA virus that is responsible for 350-400 million cases of chronic infection worldwide. Although there are 8 HBV genotypes (A-H) worldwide, the disease process is the same for all. Genotypes A&D predominate in the western world. Humans are the natural host for HBV and the virus is transmitted parenterally, thereby infecting injection drug users, men who have sex with men, healthcare workers and children born to infected mothers.

Clinically: More than 50% of HBV infections are asymptomatic, but infection classically presents as an acute hepatitis, with abdominal discomfort and jaundice. Acute HBV infection in adults resolves completely in more than 95%, with the remainder becoming chronically infected. In contrast, more than 90% of children infected perinatally develop chronic infection. Chronic HBV infection leads to fibrosis, cirrhosis (in 8-20% at 5 years), decompensated liver disease (in 20% of patients with cirrhosis at 5 years), hepatocellular carcinoma (in 2-5% of patients with cirrhosis each year), and death.

Diagnosis: HBV infection is diagnosed on the basis of detectable surface antigen (HBsAg) in the blood of infected individuals. HBsAg is the preferred marker of infection as it is present in far higher concentrations (x 1000) than HBV DNA. HBV DNA (viral load) testing is used to monitor treatment response.

NVRL Tests: HBV infection is confirmed by testing for HBsAg. HBsAg quantification testing is not performed at the NVRL. Serum/plasma specimens are therefore referred to the Virus Reference Department, Public Health England (Colindale) for testing. Acute and chronic infection are distinguished based on the presence or absence of additional serological markers, including IgM antibody to HBV core antigen (anti-HBc IgM) and HBV 'e' markers (antigen & antibody). Prior HBV infection or vaccination can be identified by the presence of anti-HBc (total) antibody and antibody to HBsAg (anti-HBs).
HBV viral load testing is available for serum and plasma samples, and genotyping / antiviral drug resistance is available on request. The rapid GeneXpert™ HBV viral load assay is available, with a TAT of 3 hours, for urgent samples or by prior arrangement.

Pathogen Specific Notes:
1: In keeping with best practice, the NVRL performs Hepatitis Delta (HDV) serology on all newly diagnosed HBsAg positive individuals
2: Hepatitis B is a vaccine-preventable infection: post vaccination testing is recommended for persons whose subsequent clinical management (e.g. immunocompromised) or occupational risk (e.g. healthcare workers) depends on knowledge of their status. Testing should be performed 2 months after the last dose of vaccine
3. WCBs with z-clot activators are not suitable for Hepatitis B (Quantitative) testing.

Hepatitis C Virus

Introduction: Hepatitis C virus (HCV) is a single-stranded RNA virus from the Hepacivirus genus of the Flaviviridae, and is the leading indication for liver transplantation in the majority of countries. The virus is divided into 6 main genotypes that have different response rates to standard antiviral therapy (pegylated interferon plus ribavirin). The virus is distributed worldwide, with some 200 million individuals chronically infected. However, with the recent development of specific anti-HCV antiviral agents, chronic HCV infection should be considered a curable disease and all infected individuals should be considered for treatment. Humans are the natural host for HCV, and transmission occurs parenterally, thereby affecting injection drug users, men who have sex with men, healthcare workers and children born to infected mothers.

Clinically: The majority of HCV infections are asymptomatic at time of acquisition, with acute HCV only rarely being identified. Only a minority of individuals resolve the infection naturally, with 60-80% progressing to chronicity. In this group, chronic hepatitis leads to fibrosis, cirrhosis, hepatocellular carcinoma, and death.

Diagnosis: HCV (in the form of either RNA or antigen) is detectable in the blood of infected individuals. HCV antibodies typically develop within 6-12 weeks of infection.

NVRL Tests: HCV serology is the preferred first-line screening test if HCV infection is suspected. HCV antigen testing identifies those patients that are viraemic, and likely to be chronically infected. HCV RNA (viral load) testing and genotyping are used to inform the decision to initiate antiviral therapy and monitor treatment responses.
Rapid (Xpert™) HCV testing is available with at TAT of 3 hours. Additional biomarker investigations (e.g. IL-28, IP-10) may be performed in individuals infected with HCV genotype 1 to aid in predicting response to novel antiviral agents. Samples for HCV genotype will not be tested if there has been a HCV genotype result within the last 6 months.

Pathogen Specific Notes:

1: Hepatitis C antigen testing is ***not*** as sensitive as RNA (viral load) testing: as such, a negative result does not exclude low level viraemia. Therefore, all individuals newly identified as HCV antibody positive & HCV antigen negative should have HCV RNA testing performed.

Hepatitis Delta [Sub viral Particle]

Introduction: Hepatitis delta virus (HDV) is in fact a sub-viral particle that relies on the presence of surface antigen from hepatitis B virus (HBV) to cause infection in humans. As such, HDV can only cause infection in those individuals who are also infected with HBV. HDV possesses a circular single-stranded RNA genome and can be divided into 8 genotypes. The 'virus' is distributed globally and humans are the natural host. Transmission occurs parenterally, leading to an increased prevalence in injection drug users.

Clinically: HDV can cause a severe acute hepatitis when co-infection with HBV occurs: the majority of these infections resolve. HDV can also superinfect those individuals already chronically infected with HBV: in these cases, chronic infection results in the majority. Of note, chronic HDV infection appears to reduce the HBV replication rate, but increases the risk of fibrosis, cirrhosis, and hepatocellular carcinoma. HDV-induced hepatitis is immune-mediated.

Diagnosis: Although HDV RNA is detectable in the blood of chronically infected individuals, serology is the preferred approach for the initial diagnosis: RNA (viral load) testing can be used to monitor treatment response.

NVRL Tests: HDV infection can be confirmed serologically (IgG & IgM), and antibody testing should be performed on all patients chronically infected with HBV. RNA testing is not performed routinely in the NVRL. Specimens are referred to the Virus Reference Department in PHE Colindale in the UK.

Pathogen Specific Notes:

1: Hepatitis B (HBV) status should be provided when requesting HDV testing. Please note that HBsAg negative samples will not be tested for HDV.

2: In keeping with best practice, the NVRL performs Hepatitis Delta (HDV) serology on all newly diagnosed HBsAg positive individuals: a separate HDV request/sample is not required

Hepatitis E Virus

Introduction: Hepatitis E virus (HEV) is a single-stranded RNA virus in its own genus, Hepevirus. Arguably, HEV can be considered the most frequent cause of acute viral hepatitis worldwide. There are four viral genotypes distributed unevenly across the globe, and in the western world, the virus has traditionally been recognised as a cause of travel-associate hepatitis, as HEV is endemic in India, SE Asia, the Middle East, and North Africa. The virus is transmitted faeco-orally, and may cause significant outbreaks due to contaminated food or water. The natural reservoir for genotypes 1&2 is not yet known: genotypes 3&4 can be considered zoonotic infections originating in swine.

Clinically: HEV infection typically presents with pain, fever, jaundice, nausea and vomiting. The infection is usually self-limiting although severe disease progressing to acute liver failure occurs in about 1%. HEV is not classically considered a cause of chronic viral hepatitis: however, persistent infection can occur in the immunocompromised host. Mortality rates do appear to be higher in pregnancy but the underlying reason for this feature has not been definitively elucidated. The phenomenon is not seen with all HEV genotypes, nor is it consistently across all geographic regions.

Diagnosis: HEV RNA can be detected in stool samples from infected individuals, and also in blood during early infection: however, serology (IgM) is the preferred approach as antibody is usually present by the time of clinical presentation.

NVRL Tests: HEV antibody testing, RNA testing, and genotyping are available in the NVRL. Genotyping is not routinely indicated for patient management, but may be used to distinguish between infections acquired overseas (typically types 1&2) or locally (typically types 3&4).

Pathogen Specific Notes:

1: The first-line Hepatitis screen in the NVRL comprises HAV IgM, HBsAg, and HCV antibody: HEV serology forms part of the second-line, with CMV and EBV

Herpes Simplex Virus

Introduction: Herpes Simplex virus (HSV) is a double-stranded DNA virus in the Simplex virus genus of the Alphaherpesvirinae subfamily of the Herpesviridae. There are two HSV types: 1 and 2. HSV-1 is classically associated with oral ulceration (cold sores) and encephalitis, while HSV-2 is classically associated with genital ulceration and meningitis (as a complication thereof). However, both viruses can cause all associated syndromes. Humans are the natural reservoir for HSV and the virus is transmitted via direct contact with saliva or through sexual activity. Both HSV-1 and HSV-2 can be found globally. HSV remains latent for life in infected individuals & may be shed asymptomatically during periods of intermittent reactivation.

Clinically: The majority of primary HSV infections are asymptomatic. In symptomatic individuals , orogenital ulceration (developing from characteristic vesicular lesions) typically is the presenting feature. Complications affecting the eye, brain (encephalitis/meningitis), and skin (erythema multiforme) are well described.. In immunocompromised patients, HSV may also cause pneumonitis or hepatitis.

Diagnosis: HSV can be detected by PCR in most body fluids, depending on the clinical presentation. Swabs of lesions or vesicular fluid are the preferred sample type in orogenital ulceration. CSF is the specimen of choice in cases of encephalitis, and respiratory secretions should be tested if HSV pneumonitis is suspected. HSV serology is not indicated for routine diagnosis of infection but type-specific serology may be useful in distinguishing between primary and secondary infection (where a contemporaneous PCR result already exists). HSV DNA can also be detected in urine, conjunctival swabs, rectal swabs, CSF, and blood samples from infected neonates.

NVRL Tests:
Swabs from affected sites for PCR - sample of choice for acute infection
CSF for PCR – encephalitis
HSV IgG and type specific serology – pregnancy / primary infection during pregnancy
Blood for PCR– neonatal HSV (by request. EDTA preferable to serum)
BAL – HSV pneumonitis - PCR (by request)

HSV IgM serology is not available at the NVRL

Herpes simplex (neonatal) is now a notifiable infection in Ireland (December 2018)
HSV infection in the neonate is rare but can lead to potentially devastating outcomes.

Neonatal HSV is associated with high morbidity and mortality. The presentation is classified into 3 main categories: disseminated disease and central nervous system (CNS) disease account for 70% of presentations; and skin, eyes, and mouth (SEM) disease accounts for the other 30%. Disseminated disease has the highest mortality rate, and CNS disease has the most significant morbidity related to neurologic injury.

In neonates, infection is mainly acquired during delivery with some acquired postpartum and rarely due to intrauterine infection. Risk of peripartum infection is dependent on whether maternal infection is primary or recurrent and on the timing of maternal infection.

The case definition for a laboratory confirmed case with or without clinical illness in a child age ≤42 days of age includes: virus isolation, or detection of HSV DNA, from an appropriate clinical specimen (CSF, blood, skin or other tissue) or histologic evidence of HSV infection from an autopsy in the case of death. A probable case is defined as a clinically compatible illness in a child age ≤ 42 days without other known causes of infection [1].

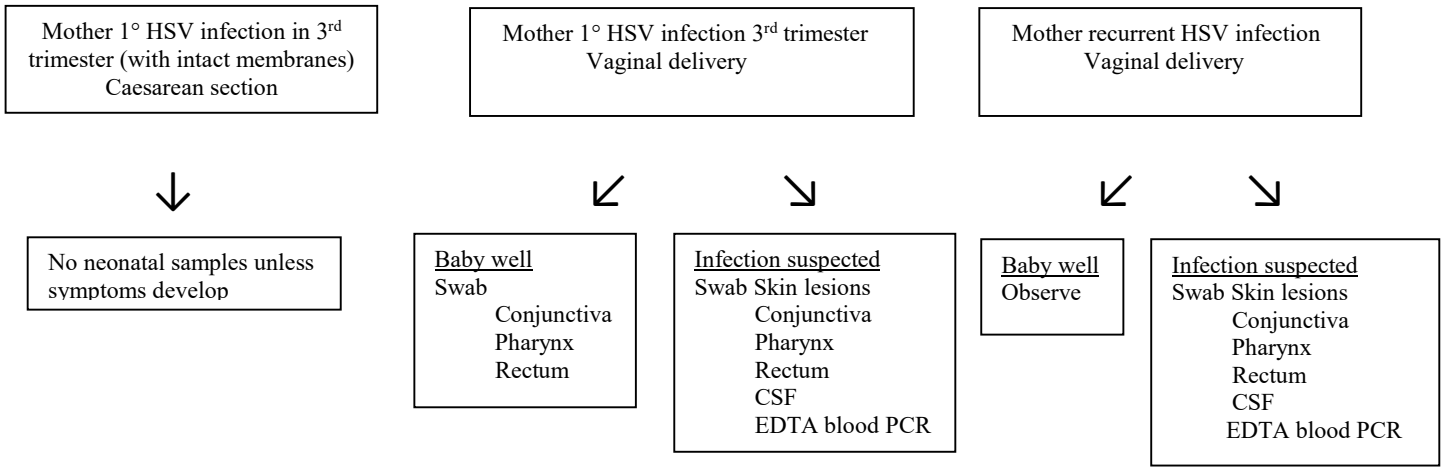
Diagnosis in Neonates: Swabs from the conjunctivae, mouth, nasopharynx, and anus should be sent for HSV PCR, as should swabs of any skin vesicles that are present. EDTA blood should also be collected and tested by PCR. In the case of CNS disease, a CSF and blood should be sent for HSV PCR. Serology (HSV IgG) is only useful in demonstration of maternal HSV infection status (to differentiate primary infection from reactivation) but is not helpful in testing neonatal samples [2].

References:

1. http://www.hpsc.ie/a-z/other/neonatalherpes/casedefinition/
2. https://www.hse.ie/eng/services/publications/clinical-strategy-and-programmes/national-laboratory-handbook-volume-1.pdf

Please see testing algorithm below if neonatal HSV infection suspected.

If neonatal HSV infection suspected:



Human Herpesvirus Type 6

Introduction: Human herpesvirus type 6 (HHV-6) is also a double-stranded DNA virus of the family Herpesviridae, but belongs to the Roseolovirus genus of the Betaherpesvirinae subfamily. There are two HHV-6 variants (A&B), although there is no clinically significant difference between the two. The majority of children are infected with HHV-6 in the second year of life. Humans are the natural host for HHV-6 and the virus is distributed globally. HHV-6 is transmitted in saliva (salivary glands are the main site of viral replication) or vertically from mother to child. Of note, HHV-6 has the ability to integrate into the host genome: as such, clinical cases in which very high viral loads are detected should be further evaluated for evidence of integration.

Clinically: HHV-6 is classically associated with exanthema subitum (6^{th} disease) and febrile convulsions in otherwise healthy children. More recently however, it has become apparent that HHV-6 can cause significant morbidity in immunocompromised populations (especially post-transplant) in the form of delayed engraftment post-HSCT or end organ disease (including encephalitis).

Diagnosis: HHV-6 DNA can be detected by PCR in the blood, saliva, and CSF of infected individuals, depending on the clinical syndrome. PCR is the test of choice in the transplant setting. Serology is available but not routinely performed, as the majority of infections occur in childhood and are self-limiting.

NVRL Tests: HHV-6 DNA testing is available in the NVRL

Pathogen-Specific Notes:

1: HHV-6 DNA testing is by request only in immunocompromised adults and children <3 yrs of age (with the exception of neonates).

2: Chromosomal integration of HHV-6 occurs in 1-3% of the population. In the setting of a high HHV-6 viral load ($<10^6$ copies/ml), comparison with a cellular marker of known quantity (typically ß-globin) can be performed to confirm/exclude integration. In immunocompetent individuals, the HHV-6 DNA load in whole blood and serum can also be used; in both cases the result will be characteristically high in viral integration, but the concentration will be about 50-fold lower in serum

Human Herpesvirus Type 7

Introduction: Human herpesvirus type 7 (HHV-7) is the second member of the Roseolovirus genus of the Betaherpesvirinae subfamily, and as such is also a double-stranded DNA virus. There is only a single genotype of HHV-7, and infection with HHV-7 is also almost universal by the fourth year of life. Humans are the natural host for HHV-7 and the virus is distributed worldwide. As with HHV-6, the virus is transmitted in saliva.

Clinically: HHV-7 is typically associated with febrile convulsions and exanthema subitum, although infection can also be asymptomatic.

Diagnosis: HHV-7 DNA can be detected in CSF, saliva, and blood of infected patients. Serology is also available, but not routinely performed.

NVRL Tests: There is at present no test for HHV-7 available in the NVRL. Samples are referred to the Virus Reference Department, Public Health England, Colindale. Plasma is the preferred sample type at PHE Colindale, although serum can also be tested.

Human Herpesvirus Type 8/ Kaposi Sarcoma Associated Herpesvirus

Introduction: Human herpesvirus type 8 (HHV-8), like all Herpesviridae, is another double-stranded DNA virus: it belongs to the Rhadinovirus genus in the Gammaherpesvirinae subfamily. HHV-8 is divided into 5 different clades that have a characteristic geographic distribution. Humans are the natural host for the virus, and the virus has a global distribution although it is uncommon in the developed world. HHV-8 is transmitted parenterally and in saliva.

Clinically: HHV-8 is – as its alternative name indicates – the causative agent of Kaposi's Sarcoma (KS), a malignancy that manifests in a variety of clinical circumstances. Classic KS affects elderly men of Mediterranean, eastern European or Jewish heritage; endemic KS occurs in east and central Africa; AIDS-associated KS is a more aggressive form of the disease that often presents with visceral involvement; and iatrogenic KS occurs in those immunosuppressed individuals who are immunosuppressed as a result of exogenous agents (mainly post organ transplantation). HHV-8 also causes primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD), both B cell lymphoproliferative disorders.

Diagnosis: HHV-8 DNA is present in the peripheral blood, effusions, and tumours of infected individuals. Serological tests are also available but are more useful in the epidemiological setting. Ultimately however, the clinical diagnosis of KS, PEL, or MCD will be made by a combination of virological and histological findings.

NVRL Tests: HHV-8 DNA testing is not available in the NVRL. Samples are referred to the Virus Reference Department, Public Health England, Colindale. Plasma is the preferred sample type at PHE Colindale, although serum can also be tested.

Human Immunodeficiency Virus

Introduction: Human immunodeficiency virus (HIV) is a single-stranded RNA virus from the Lentivirinae subfamily of the Retroviridae. As a retrovirus, HIV is characterised by its ability to reverse transcribe its RNA genome into a DNA intermediate that is then integrated into the host genome. There are two types of HIV (1&2), with HIV-1 divided further into groups (M, N, and O) with group M responsible for the global pandemic. HIV-1 (M) is then subdivided into clades (A-D, F-H, and K) that are distributed across the globe in distinct geographic patterns. Clade B is the predominant clade in the western world. The virus is transmitted parenterally & sexually, and consequently has historically been overrepresented in injection drug users, men who have sex with men, and children born to infected mothers. However, heterosexual transmission is now a significant factor in the spread of the virus. Humans are the natural host for HIV, with the original ancestor virus having been transmitted from chimpanzees in the late 19th/early 20th century. Of note, HIV-2 is rare in Ireland.

Clinically: HIV-1 is the causative agent of AIDS, causing immunosuppression through progressive destruction of CD4 T cells in infected individuals. Initial infection is associated with a seroconversion (severe glandular fever type) illness in 50-90% of individuals, followed by a prolonged asymptomatic phase. If seroconversion is missed, and the individual is not screened during the subsequent asymptomatic phase, the diagnosis of HIV is often not made until the individual presents with an (AIDS-defining) opportunistic infection, such as Pneumocystis jiroveci (formerly carinii) pneumonia, cerebral toxoplasmosis, or atypical mycobacterial infection. HIV-2 causes a similar but significantly milder disease process.

Diagnosis: HIV RNA is present in the blood of infected individuals, but may be below the limit of detection of molecular assays during the asymptomatic phase. As such, combined antigen/antibody assays are the screening test of choice for HIV. HIV genome sequencing is used to identify mutations associated with antiviral drug resistance, and the clade of the virus.

NVRL Tests: EDTA/plasma samples are required for HIV PCR testing. **These samples must be spun and frozen within 24 hours of being drawn.**
HIV-1 RNA, serology, and resistance testing (protease, reverse transcriptase & integrase), tropism testing, and genotyping will be facilitated by the NVRL, as required. Rapid Xpert™ HIV assay is available with TAT of 3 hours, for urgent samples or by prior arrangement.
Samples requesting HIV-2 testing are referred to University College, London. Separated EDTA plasma or whole EDTA bloods samples are required.

Pathogen-Specific Notes:

1: HIV RNA testing is not recommended in anti-HIV negative individuals, as non-specific (false positive) results may be generated at the lower limits of the assay

2: HIV resistance testing is not recommended in patients with a viral load of <1000 copies/ml

3: HIV-2 RNA testing is not available in the NVRL: samples are sent to UCLH NHS Foundation Trust, so it is *essential* samples are correctly labelled as HIV-2 (and not just HIV)

Human Metapneumovirus

Introduction: Human Metapneumovirus (HMPV) is a single-stranded RNA virus from the Pneumovirinae subfamily of the Paramyxoviridae. There are two HMPV genotypes (A&B) with two subgroups in each. As the name suggests, humans are the natural host for HMPV and the virus is distributed worldwide. Transmission occurs via respiratory secretions and virtually all children have been infected by 5 years of age.

Clinically: HMPV is a leading cause of respiratory illness in children, and is second only to RSV as a cause of bronchiolitis in early childhood. The most common presentation is an RSV-like infection of the lower respiratory tract, but it can also cause infection of the upper respiratory tract, otitis media, wheezing, and severe disease in immunocompromised individuals.

Diagnosis: HMPV is detectable by IF and PCR in respiratory secretions from infected patients.

NVRL Tests: HMPV molecular testing (PCR) is the test of choice. HMPV PCR is a component of the Molecular Respiratory Screen (current test code LRSC) along with Adenovirus, Influenza A,B viruses, Bocavirus, Parainfluenza, Rhino/Enterovirus, Coronavirus, SARS-CoV-2 virus, *M. pneumoniae* and *C,pneumoniae*

Human Papillomavirus

Introduction: Human papillomavirus (HPV) is a double-stranded DNA virus of the Papillomaviridae family. The family is composed of five supergroups (A-E) with subgroups (e.g. A7, A9) within each that contain the HPV types (18, 16, etc.) with which most people would be familiar. Humans are the natural host for HPV, which is distributed globally. The virus is transmitted primarily via direct contact, but mother to child transmission (during delivery) may also occur.

Clinically: HPV is the causative agent of cervical cancer, the second most common cause of cancer in women globally. HPV is also responsible for genital & non-genital warts, anal cancer, current respiratory (or laryngeal) papillomatosis, and epidermodysplasia verruciformis (a rare autosomal recessive disease). The majority of women infected with HPV do not develop cancer because HPV infection is typically a self-limiting infection. Persistent infection however with a high-risk HPV type constitutes a significant risk factor for cervical CA, and it is persistent infection in women that cervical screening programmes are designed to identify so that individuals with abnormal smears due to HPV can be closely monitored and offered therapeutic intervention before cancer develops. HPV also plays a role in oropharyngeal cancers. HPV vaccine is available through the HSE since 2010, using a quadrivalent vaccine which protects against infection with HPV 6,11,16 and 18, which are associated with 70 of cervical cancer diagnoses. School going children aged less than 15 years are the target population.

Diagnosis: The diagnosis of genital or non-genital warts is usually made on the basis of clinical examination. Cervical smear testing is at present the method of choice for diagnosing cervical HPV infection. Cytological examination initially identifies cellular abnormalities, and molecular testing for HPV DNA (or RNA) confirms the presence of HPV.

NVRL Tests: The majority of cervical HPV testing in Ireland in women is currently performed through the national cervical cancer screening programme. There is no HPV testing available in the NVRL at present. HPV virus particles can be visualised in wart samples using electron microscopy.

Human Parv 4 Virus

Introduction: Human Parv 4 virus (HP4V) is a novel human single-stranded DNA virus from the Parvoviridae family. There are 3 HP4V genotypes, and at present it is a virus for which an associated disease has not been found. As such, the clinical significance of the virus is uncertain: that being said, HP4V DNA has been detected in pooled plasma samples, blood donors, injecting drug users, and individuals with haemophilia. Some 40% of IDU and individuals with haemophilia are seropositive, with DNA detectable in 3-4%. It is not yet known whether humans constitute the natural hosts for HP4V, and the mode of transmission is also not yet established.

Clinically: No known disease association.

Diagnosis: HP4V DNA can be detected in blood samples by PCR.

NVRL Tests: There is presently no test available for HP4V in the NVRL.

Human T Cell Lymphotropic Virus

Introduction: Human T cell lymphotropic virus (HTLV) is a single-stranded RNA virus from the Deltaretrovirinae subfamily of the Retroviridae. There are four types of the virus (1-4) with 4 clades each in types 1 and 2. HTLV-1 is the most significant virus of the group from a clinical perspective. Humans are the natural hosts for HTLV, and while the virus is distributed globally, areas of increased prevalence have also been identified: these include Japan, the Caribbean, south/central America, and sub-Saharan Africa. The virus is transmitted parenterally and sexually, but most importantly vertically from mother to child. Of note, breast-feeding contributes significantly to the burden of vertically transmitted disease. HTLV infection is not common in Ireland.

Clinically: HTLV is associated with two main clinical syndromes: adult T cell leukaemia/lymphoma (ATL), and HTLV associated myelopathy (HAM). However, infection is not universally associated with disease. ATL develops in 3-5% of infected individuals after 20-30 years, and HAM leads to progressive spastic paraparesis in only 2-3% of infected individuals. The pathogenesis of HAM appears to involve CD8 cytotoxic T cells damaging CNS tissue during the process of targeting HTLV infected CD4 cells. The likelihood of HTLV disease – especially for ATL – appears to be related to the age at infection, hence the significance of interrupting vertical transmission where possible.

Diagnosis: HTLV is present in the blood of infected individuals. However, the virus is highly cell-associated and as such, proviral DNA (as against circulating free RNA) is the molecular test of choice. Serology is the preferred method for initial screening and diagnosis with molecular testing used to confirm infection and monitor response to treatment.

NVRL Tests: HTLV serology, typing, and proviral DNA (viral load) testing are available in the NVRL. Proviral tests are also referred to PHE Colindale

NB: Proviral testing requirements are listed in section 6.10, pg 28.

Influenza Virus

Introduction: Influenza virus is a single-stranded RNA virus from the Orthomyxoviridae family, of which there are four types (A-D). Influenza A is further subtyped on the basis of its haemagglutinin and neuraminidase proteins. At time of writing, there are 18 Haemagglutinin (H) types and 11 Neuraminidase (N) types. These two proteins provide influenza A viruses with their official names and typically a number of different influenza types are in circulation at any one time. In recent seasons, two influenza A viruses H1N1 & H3N2 and one influenza B virus, have been circulating. In theory, any combination of H & N types could exist. Avian influenza is an H5N1 virus, and in 2013 an H7N9 virus with high mortality emerged in China. An H10N8 virus – originally identified in 2012 – also caused its first dearth in 2013. Influenza A viruses infect humans and many different animals. The emergence of a new and very different influenza A virus with the ability infect people and have sustained human to human transmission, can cause an influenza pandemic. Influenza B viruses circulate among humans and cause seasonal epidemics. Recent data showed seals also can be infected. Influenza C viruses can infect both humans and pigs but infections are generally mild and are rarely reported. Influenza D viruses primarily affect cattle and are not known to infect or cause illness in people. Viruses that cause severe disease in poultry and result in high death rates are called highly pathogenic avian influenza (HPAI). Viruses that cause mild disease in poultry are called low pathogenic avian influenza (LPAI).
Influenza viruses are globally distributed and transmission occurs via respiratory secretions. Novel influenza viruses that emerge in birds typically have to acquire a number of genetic mutations before they can become successfully established in the human population.

Clinically: Influenza infections in humans may cause disease ranging from mild upper respiratory infection (fever and cough) to rapid progression to severe pneumonia, acute respiratory distress syndrome, shock and even death. Gastrointestinal symptoms such as nausea, vomiting and diarrhea has been reported more frequently in A(H5N1) infection. Conjunctivitis has also been reported in influenza A(H7). Disease features such as the incubation period, severity of symptoms and clinical outcome varies by the virus causing infection but mainly manifests with respiratory symptoms. The majority of individuals will recover without incident, but complications including pneumonia, myositis, otitis media, and CNS disease (encephalopathy, post-infectious encephalitis, Guillain-Barre syndrome) can occur.

Diagnosis: Influenza virus (antigen or RNA) is readily detectable in respiratory secretions (by IF or PCR respectively), but RNA is not usually detectable in blood. Given the time taken to mount a serological response, antibody testing is of little use in the diagnostic setting. However, serological testing at the population level provides important epidemiological data.

NVRL Tests: Influenza virus PCR is performed daily in the NVRL during the influenza season. Influenza subtyping is performed on all influenza RNA positive samples, and antiviral resistance testing is also available. In conjunction with the ICGP and the HPSC, the NVRL operates an annual sentinel surveillance programme for influenza from week 40 to week 20 (the accepted boundaries of the 'influenza season'). In addition, the NVRL is the World Health Organisation designated National Influenza Centre for Ireland, and provides a sample of influenza isolates from Ireland to the WHO influenza programme each year.
https://www.who.int/news-room/fact-sheets/detail/influenza-(avian-and-other-zoonotic)

Japanese Encephalitis Virus

Introduction: Japanese Encephalitis Virus (JEV) is a single-stranded RNA virus from its own subgroup of the Flavivirus genus in the Flaviviridae family. There are at least 4 JEV genotypes but these appear to have no significant clinical distinguishing features. JEV is the major arboviral cause of encephalitis worldwide, with around 50000 cases per year, of which 25% are fatal, and half of survivors are left with residual CNS deficits. The natural hosts for JEV are nesting birds, domestic animals, and mosquitoes, and the virus is endemic in 16 countries in Southeast Asia. Although the virus originated in Indonesia & Malaysia, the clinical entity was first described in Japan in 1870. Humans acquire the virus from infected mosquitoes.

Clinically: JEV infection may cause a febrile illness with headache, aseptic meningitis, or an acute progressive meningomyeloencephalitis, with CNS disease being more prevalent in those at extremes of age. In addition, JEV may persist in lymphocytes and reactivate leading to recurrent disease, especially in children.

Diagnosis: Serology is the test of choice for JEV infection. JEV RNA is typically not detected by the time of clinical presentation. However, in a situation – such as an outbreak – where infection is suspected early, JEV RNA can be detected in blood.

NVRL Tests: JEV screening serology is performed in the NVRL as part of the Euroimmune Flavivirus IFA screen. Samples that test positive on this test are referred to the Rare & Imported Pathogens Laboratory in the UK for confirmatory testing.

JC Polyomavirus

Introduction: JC Polyomavirus (JCV) is a double-stranded DNA virus, of which there are seven subtypes (1-8). The virus is named after the initials of the patient from whom it was first isolated in 1971.

Clinically: JCV is distributed worldwide with humans as natural hosts. Primary infection typically occurs in childhood and does not cause significant illness in otherwise healthy individuals. However, the virus does has the capacity to remain latent in the infected host and can reactivate either asymptomatically, or with significant morbidity in certain – almost invariably immunosuppressed – patient groups. JCV is the causative agent of Progressive Multifocal Leucoencephalopathy (PML), a demyelinating disorder of the central nervous system. In addition to being associated with advanced HIV disease and AIDS, PML has more recently been reported in patients with multiple sclerosis being treated with natalizumab (Tysabri).

Diagnosis: JCV DNA can be detected in the urine, blood, CSF and brain tissue of infected individuals. The diagnosis of PML requires the presence of JCV DNA but can be suspected on the basis of clinical and radiological findings. Whilst JCV serology is not routinely indicated to confirm infection (based on the high seroprevalence), patients with multiple sclerosis who are being considered for natalizumab (Tysabri) therapy, should be screened for JCV antibody before commencing treatment. Quantitative DNA PCR can be used to track the course of infection as well as monitor response to treatment.

NVRL Tests: JCV DNA (PCR) testing is performed in the NVRL on blood, urine, and CSF. JCV antibody testing is not currently (January 2019) available in the NVRL.

KI Polyomavirus

Introduction: KI Polyomavirus (KIV), named for the Karolinska Institute in which it was first identified, was discovered in 2007 as part of a molecular search for unknown viruses in respiratory samples. As with JCV and BKC polyomaviruses, KIV is a double-stranded DNA virus that is distributed globally, and for which humans are presumed to be the natural host. Seroprevalence studies suggest that the majority of infections occur in early childhood.

Clinically: Although KIV has been detected in respiratory samples in institutions worldwide, its definitive capacity to cause disease remains unproven, in part because of its frequent association with other viral pathogens. Consequently, at present routine testing for KIV in clinical samples is not indicated.

Diagnosis: KIV DNA can be detected in the respiratory samples of infected individuals.

NVRL Tests: There is currently no test available for KIV in the NVRL.

La Crosse Virus

Introduction: La Crosse virus (LACV) is a single-stranded RNA Bunyavirus that was first discovered in La Crosse, Wisconsin in the US in 1963. LACV is widely distributed in the US, although most common in the mid-west, and prior to the introduction of West Nile Virus, was responsible for the majority of cases of arbovirus encephalitis in the US. Small mammals including chipmunks and squirrels are the natural reservoir for LACV and the virus is transmitted to humans by infected mosquitoes.

Clinically: The majority of human infections with LACV are subclinical, but it can also cause a febrile illness with transient CNS signs. The classical association is with encephalitis, which occurs in <1% of individuals, although this rate may be higher in children. LACV infection is most commonly seen in forest workers or children who play in woodland areas.

Diagnosis: Detection of IgM by serological testing is the method of choice for confirming LACV infection, although the virus can also be retrieved from the brains of individuals with encephalitis.

NVRL Tests: LACV is not tested at the NVRL. Specimens are therefore referred to the Rare & Imported Pathogens Laboratory in the UK for testing.

Lassa Virus

Introduction: Lassa virus (LV) is a single-stranded RNA virus from the Arenaviridae family that takes its name from the town in Nigeria in which it was discovered in 1969. The haemorrhagic fever virus is endemic in several regions of West Africa, although the habitat of its natural host – the multimammate rat – is more widespread. Human infection primarily occurs following exposure to the urine of infected animals, and human-to-human transmission may also result from contact with blood, tissue, secretions, or excretions of an infected individual. As a result, patients should ideally be managed in isolation units, and strict procedures put in place for the handling & disposal of body fluids & excreta.

Clinically: The majority (80%) of LV infections in endemic areas are mild or asymptomatic. However, the remaining 20% develop a systemic febrile illness that may progress to systemic shock, with CNS involvement and severe haemorrhagic disease. Whilst the mortality at this late stage of disease is in the region of 50%, the overall mortality for LV infections is only in the region of 1%.

Diagnosis: LV can be detected in the blood and urine (by PCR) of infected individuals. However, serology (IgM) is the mainstay of diagnosis. Lassa virus is a biosafety level 4 pathogen.(BL4)

NVRL Tests: LV PCR (Qualitative) is performed at the NVRL. Samples are referred to the Rare & Imported Pathogens Laboratory in the UK for confirmatory/supplementary testing*.

***Note:** Viral Haemorrhagic fever (VHF) testing is only done by prior arrangement with clinical team and as per guidelines at:
www.hpsc.ie/hpsc/A-Z/Vectorborne/ViralHaemorrhagicFever/Guidance/
The NVRL must be notified before dispatch from hospital of any suspect VHF specimens. Please contact the laboratory on 01-7161321/7164440.

Leptospira interrogans

Introduction: Leptospira are motile spirochetes that are found worldwide. The species *Leptospira interrogans* includes all human pathogens of which there are many serotypes. The primary reservoirs of infection are wild mammals, and rats are the commonest worldwide source. Transmission occurs when people come in contact with infected animal urine through activities such as swimming, farming and canoeing. Humans are incidental hosts and onward transmission is rare.

Clinically: The incubation period is 7-12 days. Leptospirosis is a biphasic disease with initial septicaemia lasting 4-7 days characterised by an influenza-like illness. Following a 1-3 day period of improvement, the secondary phase is characterised by immune phenomena such as vasculitis and aseptic meningitis. Weil's disease is a severe form of leptospirosis associated with jaundice and acute renal failure which occurs in 5-10% of cases.

Diagnosis: Serology is the mainstay of diagnosis for Leptospirosis.

NVRL Tests: The NVRL offers a Leptospira IgM screening test for the diagnosis of current infection: however, cross-reactivity can occur resulting in false positive results. Reactive samples are therefore referred to the Leptospira Reference Unit (LRU) in Porton Down in the UK for confirmation. Additional convalescent serum samples may be requested to identify the serotype of the infecting organism. In the early stages of infection Leptospira IgM serology may be negative. Consequently, if there is a high index of clinical suspicion for Leptospira infection and initial serology results are negative, a follow up sample in 7-10 days may be useful. The NVRL clinical team is available on (01) 716 4401 for further discussion if required.

Lymphocytic Choriomeningitis Virus

Introduction: Lymphocytic choriomeningitis virus (LCMV) is a single-stranded RNA virus from the Arenaviridae family. There is a single LCMV genotype distributed worldwide, wherever its natural host – the common house mouse – is capable of residing. Human infection typically occurs following exposure to the urine of infected animals (typically through aerosolisation of contaminated dust), but the virus can also be transmitted vertically from mother to child. Human seroprevalence rates of up to 9% have been reported.

Clinically: LCMV is classically associated with aseptic meningitis, although the majority of infections are asymptomatic. In those who develop meningitis, the disease course is usually biphasic with an initial influenza-like illness followed by a few days of apparent recovery, at which time the CNS manifestations present. Some 20% of meningitis cases can progress to a fatal encephalomyelitis. However, the overall mortality for LCMV is <1%. Intrauterine infection with LCMV can also occur, resulting in birth defects or miscarriage.

Diagnosis: LCMV RNA can be detected in blood (by PCR) during the febrile phase of the illness, and in CSF later if disease progresses. Serology is also useful diagnostically, as the seroprevalence of LCMV is low, and the CNS stage of the disease may be immune-mediated.

NVRL Tests: There is no test for LCMV currently available in the NVRL. Paired serum and CSF samples for testing are referred to the Virus Reference Department, Public Health England, Colindale.

Measles Virus

Introduction: Measles is a single stranded RNA virus in the Morbillivirus genus of the Paramyxoviridae family. There are 8 measles virus (MV) clades (A-H) containing different genotypes. These different genotypes have distinct global distribution patterns and can be used in certain situations to track outbreaks and distinguish between endemic and imported MV infections. Humans are the natural hosts for MV and the virus is distributed worldwide, with an increased number of infections in winter and spring in the northern hemisphere. The virus is transmitted by droplets and aerosols, and is one of – if not – the most infectious human pathogens known, with a reproductive number of 11-18. By way of comparison, the reproductive number for seasonal influenza is 1.1-1.5. Measles is a vaccine preventable disease. During 2000– 2018, measles vaccination prevented an estimated 23.2 million deaths. Global measles deaths have decreased by 73% from an estimated 536 000 in 2000* to 142 000 in 2018.

Clinically: MV infection causes measles, a syndrome that presents with cough, coryza, conjunctivitis, and a classical maculopapular rash that begins in the post-auricular area and the forehead and spreads down to the face, neck, trunk, and extremities. While the majority of individuals recover without incident, complications can occur. The most common complications are otitis media (7-9%), pneumonia (1-6%), diarrhoea (8%), and convulsions (0.5%). More rare complications include encephalitis (0.02-0.1%) and sub-acute sclerosing panencephalitis (SSPE). SSPE is a rare fatal progressive degenerative brain disease (with an incidence of 1 in 10000 MV cases) that occurs 6-8 years following primary infection, and represents a persistent MV infection. The likelihood of SSPE is increased if primary MV infection occurs <2yrs of age, and in certain ethnic groups (Papua New Guinea), suggesting a role for host genetic and immune system factors in pathogenesis.
NVRL is part of WHO European Regional Measles/Rubella Laboratory Network, being the National Reference Laboratory for Measles and Rubella.

Diagnosis: Measles virus RNA can be detected (by PCR) in saliva in the first 5 days after rash onset. IgM is also detectable in oral fluid but appears later, at around 5-7 days. IgM is also detectable in blood, and MV can be cultured from respiratory secretions and urine.

NVRL Tests: Oral fluid is the most convenient sample for the confirmation of MV infection, on account of the non-invasive nature of the test. RNA testing is performed on oral fluid. Measles virus IgG testing is available for occupational health screens for healthcare workers. However, post-vaccine testing for Measles IgG is not routinely recommended. Measles virus sequencing and genotyping are also available for epidemiological investigations.

Pathogen-Specific Notes:

1: Measles virus RNA is routinely performed on oral fluid samples collected within 5 days of rash onset (if this information is provided on the request form)
2: Respiratory samples may also be processed following discussion with NVRL Clinical Team (01-7164401)

WHO link: https://www.who.int/news-room/fact-sheets/detail/measles

Merkel Cell Polyomavirus

Introduction: Merkel Cell Polyomavirus (MCPyV) is a double-stranded DNA virus of the Polyomaviridae family. There is at present only one known MCPyV genotype, which was discovered in 2008.

Clinically: MCPyV is strongly implicated as the causative agent of Merkel Cell Carcinoma, a rare but highly aggressive form of skin cancer. The classic presentation of MCC is defined by the acronym AEIOU (asymptomatic/non-tender, expanding rapidly, immune suppression, older than 50 years, and ultraviolet-exposed site on a person with fair skin). MCPyV DNA is integrated into chromosomal DNA of malignant tissue. The precise mode of transmission for MCPyV is has not been definitively established. However, viral DNA can be retrieved from the skin of healthy adults, and has also been detected in human sewerage.

Diagnosis: MCC is diagnosed histologically, but MCPyV DNA can be detected in tumour tissue by PCR.

NVRL Tests: No testing is available

Middle East Respiratory Syndrome Coronavirus (MERS CoV)

Introduction: Middle East Respiratory Syndrome Coronavirus (MERS-CoV) was first isolated from a patient with severe respiratory illness in Saudi Arabia in 2012. The second case was reported in a patient from the UK who was repatriated from Qatar with a similar presentation. The virus itself is closely related to Bat coronaviruses and appears to have been transmitted from bats to humans via dromedary camels as an intermediate host. The virus appears to have circulated widely in camel populations across the Middle East: why the virus has now started to infect humans is not definitively known.

Clinically: As the name suggests, MERS-CoV causes a severe respiratory syndrome that has also been associated with renal failure. At the time of writing (March 2014), there have been more than 185 cases reported with more than 80 deaths, yielding a mortality rate of >40%. Human to human transmission has been reported in clusters of cases but is not sustained and does not appear to be widespread.

Diagnosis: MERS-CoV is detectable in the respiratory secretions of infected individuals by PCR.

NVRL Tests: Molecular testing (PCR) for MERS-CoV is available by arrangement in the NVRL.

Pathogen Specific Notes: If MERS-CoV infection is suspected, public health should be notified prior to requesting laboratory testing. Please refer to the Health Protection Surveillance Centre website for the most up to date guidance www.hpsc.ie

Molluscum Contagiosum Virus

Introduction: Molluscum Contagiosum virus (MCV) is a double-stranded DNA virus from the Molluscipoxvirus genus of the Poxviridae family. Four subtypes of the virus have been described, but they are of no particular clinical significance.

Clinically: The characteristic lesion of MCV begins as a small papule that develops into a smooth dome-shaped (2-5mm), pearly or flesh-coloured nodule that is typically umbilicated. A cheesy, off-white or yellowish material is easily expressed from lesions. Individuals usually present with between 1 and 20 lesions, but there may be hundreds. MCV does not cause systemic infection, but the skin lesions may be disseminated: this presentation is more common in the immunocompromised host.

Diagnosis: The diagnosis of Molluscum Contagiosum is usually made clinically. However, MCV DNA can be readily detected in lesions by PCR, and the virus can also be seen by EM in the papular discharge and lesion tissue.

NVRL Tests: EM is available in the NVRL for confirmation of the diagnosis of MCV.

Monkeypox Virus

Introduction: Human Monkeypox infection (HMI) is a systemic viral exanthematous zoonosis. Monkeypox virus (MPXV) is a highly pathogenic enveloped DNA virus from the Orthopoxvirus genus of the Poxviridae family, which includes the virus responsible for smallpox (variola). It is characterised in Ireland as a Biosafety Level 3 pathogen.

First identified in 1958, the virus is found in some small animal & rodent populations in remote parts of Central and West Africa and causes occasional limited outbreaks in communities and travellers. Possibly as a result of waning immunity to Smallpox which was eradicated in 1979, cases and geographical extent of HMI have been increasing in recent decades. Given that HMI is a re-emerging disease, whose epidemiology is evolving, the WHO has identified MPXV as being the most important human orthopoxvirus infection following the eradication of smallpox.
In 2022 increased numbers of cases of HMI were reported outside of Africa with no known travel to the region. There are two clades of Monkeypox virus: West African monkeypox and Congo Basin monkeypox. The 2022 outbreak is caused by the milder West African clade, https://www.hpsc.ie/a-z/zoonotic/monkeypox/guidance/

Clinically: Monkeypox (MPX) generally spreads through respiratory droplets, direct contact with bodily fluids or sores of an individual infected with MPXV, or direct contact with materials that have touched bodily fluids or sores of an infected individual such as bed linen or towels. In addition, the virus can be inhaled from airborne particles from aerosol generating procedures or the agitation of contaminated fomites. Those in close contact with an infected individual are more at risk of infection: including household members, sexual partners and healthcare workers. The risk of spread within the community is, in general, very low.
Symptoms of monkeypox virus infection include an itchy rash that starts as raised red spots that quickly change into little blisters. It usually develops within 1 to 3 days of the start of a fever (>38.5) but some individuals only have a rash. Classically, the rash starts on the face, and spreads to the mouth, palms of hands and soles of the feet. However, following sexual contact, the rash may be found initially in the anogenital area.
With individuals infected in the 2022 international outbreak, the rash in the anogenital area may be atypical, and may be the main symptom. The rash goes through different stages before finally forming scabs which later fall off. (See images of these stages at: www.hpsc.ie/a-z/zoonotic/monkeypox/factsheet)

Diagnosis: Perform a clinical assessment, including a test for Monkey Pox (MPXV) DNA using one standard viral swab in viral transport medium. The swab should be taken from a cutaneous lesion either ulcer or vesicular fluid if present. If there are concerns that patient is presenting during the prodromal stage and there are no cutaneous lesions, a throat swab may be taken instead. A negative result for the throat swab does not rule out MPXV and clinical correlation is advised and a follow up swab sample is required if lesions develop.

NVRL Tests: NVRL tests in relation to MPXV infection include 2 PCR based assays: one is a commercial 'pan-orthopoxvirus DNA' assay and the second is an in-house, laboratory-developed West African clade MPXV specific assay. These tests are performed daily – Monday to Friday.
Swab samples only must be transported to the laboratory as Category A pathogens. Double bag the sample at the point of collection in the clinic setting. The referring clinician should

inform the local microbiologist, the local Public Health department and the NVRL of probable samples for MPX investigation.

Only once a 'not detected' result is obtained may further samples from a patient be sent for other tests performed at the NVRL

Mumps Virus

Introduction: Mumps virus is a single-stranded RNA virus from the rubulavirus genus of the Paramyxoviridae family. Although there is only a single mumps serotype, there are 12 (A-L) genotypes. The genotypes are of little clinical significance but do facilitate the identification of imported mumps cases or epidemiologically linked mumps cases. Humans are the natural reservoir for Mumps virus (MV) and the virus is distributed globally. Mumps cases typically peak in February and March in temperate zones. Transmission occurs via droplet.

Clinically: The classic presentation for mumps is parotid swelling (75% of cases are bilateral); although in countries without immunisation programmes, mumps virus is also a leading cause of aseptic meningitis, although not all patients with abnormal CSF findings are symptomatic. Recognised complications (although the term systemic manifestations is probably more accurate) of MV infection include orchitis (20-30%), oophoritis (5%), and pancreatitis (<5%). The lack of a recognised exposure/source in individuals with confirmed mumps suggests that between 20% & 40% of MV infections may actually be asymptomatic.

Diagnosis: Mumps virus can be detected (by culture or PCR) in the oral fluid, throat swabs, CSF, and urine of infected individuals. MV specific IgM is also present in oral fluid, but can take 5-7 days post onset of symptoms to appear. MV specific IgM in blood can also take 1-3 weeks to become detectable in some cases.

NVRL Tests: Oral fluid is the specimen of choice for the investigation of mumps cases in the NVRL for RNA. IgG and IgM can also be performed on blood. MV sequencing is not routinely indicated but is available for the investigation of outbreaks or suspected imported infections (in conjunction with public health).

Murray Valley Encephalitis Virus

Introduction: Murray Valley Encephalitis Virus (MVEV) is a single-stranded RNA flavivirus, of which there are four (I-IV) distinct lineages. The name derives from the valley of the Murray River in SE Australia where the infection was first discovered. It is an uncommon – although potentially fatal – cause of human disease. Birds and mosquitoes are the natural hosts for MVEV and to date; the virus has been confined to Australia and New Guinea. The virus is transmitted to humans by infected mosquitoes.

Clinically: Although the majority of human infections are symptomatic, infected individuals may also experience a febrile illness with headache & meningism that may progress to encephalitis and coma. Mortality in those who reach the latter stages of CNS disease ranges from 20-40%.

Diagnosis: Viral RNA may be detectable in blood early in the course of the infection, but serology (for IgM) is the diagnostic method of choice.

NVRL Tests: MVEV testing is not routinely performed in the NVRL. Samples from patients with the appropriate travel history and clinical syndrome are referred to the Rare and Imported Pathogens Laboratory in the UK.

Mycoplasma genitalium

Introduction: *Mycoplasma genitalium* (MG) is a sexually transmitted bacterial infection. It is estimated that MG is the second-most prevalent STI next to *Chlamydia trachomatis* infection, with prevalence rates ranging from 0.4% in the United States to 4.5% in some European countries.

Clinically: MG infection may present as urethritis in men and is associated with pelvic inflammation disease and cervicitis in women. However, the full spectra of clinical manifestations following infection are not yet fully elucidated. In addition, many MG infections may be asymptomatic

Diagnosis: MG is a slow-growing organism and culture can take up to 6 months. Therefore, Nucleic Acid Amplification Techniques (NAAT) testing is the preferred method for MG detection. MG may be detected by PCR in urine and anogenital swabs of infected individuals. Further follow-up testing for resistance markers is recommended as antimicrobial resistance presents a major challenge to the treatment of MG infection.

NVRL Tests: MG NAAT testing is available by request using the Aptima *Mycoplasma genitalium* Assay (Hologic). Please note: Throat or oral swabs are not suitable for Mycoplasma *genitalium* testing. Any throat or mouth swabs will not be tested. Please refer to the NVRL website (https://nvrl.ucd.ie) for information on ordering Aptima collection devices. All specimens with detectable M. genitalium will be referred for confirmation and antibiotic resistance testing at.

PHE Colindale
61 Colindale Ave
London NW9 5HT

Mycoplasma pneumoniae

Introduction: Mycoplasma pneumonia is a bacterium that causes respiratory tract infection and is a cause of atypical pneumonia. Infections occur all year round but peak occurrence is in winter. Severe disease is most often seen in younger age groups from age 5 to young adulthood. Infection is spread by droplet transmission and the incubation period is 2-3 weeks. Reinfection is possible.

Clinically: Presentation is usually insidious with influenza-like symptoms. Pneumonia may develop with haemoptysis and/or purulent secretions. Multiple lobes may be involved but without consolidation, and CXR findings are often more dramatic than clinical presentation suggests. Rare complications include pleuritis, pneumothorax, lung abscess, haemolytic anaemia (secondary to cold agglutinins), thrombocytopenia, arthritis, rashes e.g. erythema multiforme, & Guillain Barre Syndrome. Most complications are immune-mediated with the exception of neurological complications such as meningoencephalitis, which are thought to be due to direct invasion.

Diagnosis: Mycoplasma pneumonia lacks a rigid cell wall and has strict growth requirements: as a result it is not routinely identified by culture or microscopy. Serology & molecular testing therefore are the preferred means for diagnosis.

NVRL Tests: Mycoplasma IgM and IgG are available in the NVRL. Note the IgM response may be attenuated (lessened) in repeat infections. In early infection a weakly reactive or equivocal IgM result may be reported and a follow up (convalescent sample) in 7-10 days may be useful to demonstrate a rise in IgM titres and/or seroconversion of IgG to confirm the diagnosis.
Mycoplasma pneumoniae molecular test is available in the NVRL as part of the respiratory screen.

Neisseria gonorrhoeae

Introduction: Neisseria gonorrhoeae (NG) is a gram-negative bacterium, which only affects humans and, and is the causative agent of the sexually transmitted infection Gonorrhoea. There is a worldwide distribution of infection.

Clinically: Lower genital tract infection may be asymptomatic or cause urethritis with purulent discharge and dysuria in men and endocervicitis with PV discharge, itch and dysuria in women. Infection of the pharynx and/or rectum can occur and may be asymptomatic. Complicated infection may lead to epididymitis/epididymorchitis in men or pelvic inflammatory disease and tuboovarian abscess in females. Disseminated gonococcal (GC) infection occurs in <1% of cases and presents with symptoms such as fever, arthralgia, rash, septic arthritis, endocarditis and/or meningitis. Neonates acquiring infection intrapartum may present with ophthalmia neonatorum and/or disseminated infection. Conjunctivitis can also occur in adults.

Diagnosis: Laboratory diagnosis of NG infection is based on nucleic acid amplification testing (NAAT) and/or by culture of the bacterium. Culture should be taken in all GC cases diagnosed by NAAT prior to antibiotics being given so that susceptibility testing can be performed and resistant strains identified. NG infection can also be identified by the presence of gram-negative diplococci within leucocytes in swabs of urethral discharge.

NVRL Tests: Combined *Chlamydia Trachomatis* (CT) and NG testing is performed in the NVRL. Specimen collection advice is included in the APTIMA kits provided by the NVRL. Three types of specimen collection device (SCD) are available: vaginal swab; Unisex/endocervical swab; and urine. Following specimen collection, the SCD are stable at room temperature for 60 days.

Pathogen-Specific Notes:

1: Only specimens collected in APTIMA collection devices can be tested in the NVRL.

2: Please refer to section 5.5 Specimen Collection for information related to the APTIMA collection devices. Please pay particular attention to the sample level required in the SCD.

3: A ***test of cure*** is recommended in all GC cases: please refer to BASHH Guidelines

Nipah Virus

Introduction: Nipah virus (NV) is a single-stranded RNA virus from the Henipavirus genus of the Paramyxoviridae family. There are two genotypes, discovered in Malaysia and Bangladesh in 1999 and 2004 respectively. The natural host for NV is the pteropid bat (flying fox) but humans typically become infected through contact with the intermediate host, swine. Nipah virus is distributed throughout Southeast Asia (Malaysia, Singapore, India, Bangladesh, and Cambodia) and is named for the village in Malaysia where the first isolate was recovered. Human to human transmission has been reported during outbreaks, but not in the healthcare setting, suggesting that standard precautions and hygiene are sufficient to prevent spread of the virus.

Clinically: Nipah virus has been responsible for outbreaks of encephalitis across SE Asia and encephalitis (which may be relapsing) is its most prominent clinical manifestation. The mortality in hospitalised patients may be as high as 30%.

Diagnosis: NV is present in the respiratory secretions and urine of infected individuals, but serology is the diagnostic method of choice.

NVRL Tests: Testing for Nipah virus is not routinely performed in the NVRL, but samples from patients with the appropriate travel history and clinical syndrome are referred to the Rare and Imported Pathogens Laboratory in the UK.

Norovirus

Introduction: Norovirus is a single-stranded RNA virus of the Caliciviridae family. The name derives from Norwalk, Ohio in the US where the virus caused an outbreak of gastroenteritis in an elementary school in 1968. There are 5 norovirus genogroups with multiple genotypes within each. The typings are of little clinical significance but are used routinely for epidemiological purposes to monitor the emergence and spread of novel predominant strains over time. Humans are the natural host for norovirus and transmission occurs via the faeco-oral route or by aerosol.

Clinically: Norovirus is the second most important cause of viral gastroenteritis worldwide. The classic presentation is of sudden onset vomiting and diarrhoea that lasts for 24-48 hours and then resolves. Persistent infection can occur in immunocompromised individuals, and some 30% of infections are thought to be asymptomatic.

Diagnosis: Norovirus can be detected in the stool and vomitus of infected individuals, typically by EM, under which it appears as a small, round, structured virus (SRSV), or PCR. PCR is far more sensitive. The required viral burden for a positive EM finding is $>10^6$ viral particles per ml of faecal material. PCR also has the capacity to distinguish between genogroups.

NVRL Tests: The NVRL performs PCR routinely for Norovirus, although EM is also available. Frequency of testing is increased during the winter ('winter vomiting virus') but is performed all year round.

Orf Virus

Introduction: Orf virus is a double-stranded DNA virus from the Parapox genus of the Poxviridae family. Orf is a zoonotic infection that is maintained in nature in sheep and goats.

Clinically: Human infections with Orf are an occupational hazard for farmers, abattoir workers, and veterinarians, with the highest prevalence seen during lambing and calving season. The classic Orf lesion begins as an erythematous papule that progress to a 'target' stage (red centre surrounded by a white halo, with an outer inflamed halo). The lesion then becomes nodular, papillomatosis, and may 'weep'. Typically the lesion then resolves following a crusting stage, although they may persist for some weeks before resolution. Single lesions are the norm, but multiple primary lesions may occur. Systemic manifestations are uncommon and the lesion is not particularly painful.

Diagnosis: Clinical diagnosis in the right setting (with the appropriate exposure history) is typically sufficient, but virus is readily detectable (EM or PCR) in lesion extracts.

NVRL Tests: Orf virus infection is diagnosed by EM in the NVRL, sample types: Skin, Tissue, vesicle fluid and scrapings.

Parainfluenza Virus

Introduction: Parainfluenza virus (PIV) is a single-stranded RNA virus, the four major types of which span two genera of the Paramyxovirinae subfamily of the Paramyxoviridae family of viruses: PIV 1&3 are in the Respirovirus genus, and PIV 2&4 are in the Rubulavirus genus. Of the four major types (1-4), subtypes exist in PIV 1, 3, and 4 although they are of little clinical significance. Humans are the natural reservoir for PIV infection and the viruses are distributed worldwide. Typically, PIV 1 causes biennial peaks from September to December, whereas PIV 3 causes annual outbreaks in Spring/Summer time. Transmission of virus occurs via close contact and surface contamination.

Clinically: PIV constitute one of the leading causes of hospitalisation for acute respiratory illness worldwide, accounting for ≤ 30% of hospitalisations in paediatric patients. PIV 1 is classically associated with acute laryngotracheobronchitis (croup) and PIV 3 with bronchiolitis and pneumonia. Hospital outbreaks of PIV have also been reported, predominantly in immunocompromised patient groups when persistent (unrecognised) shedding may occur. PIV infection can also cause bronchiolitis obliterans in lung transplant recipients.

Diagnosis: PIV is readily detectable (by IF and PCR) in the respiratory secretions of infected individuals.
NVRL Tests: PIV molecular testing is the test of choice in the NVRL. Immunofluorescence is available on request only

Parechovirus

Introduction: Human Parechovirus (HPeV) is a single-stranded RNA virus from the Picornaviridae family that has relatively recently been granted its own genus, having previously been considered one of the Enteroviruses. There are 8 recognised HPeV types with a further 6 proposed, but HPeV 1, 2, and 3 accounts for most clinically recognised infections. Humans are the natural hosts and the viruses are distributed globally. Transmission occurs via the respiratory and faecal-oral routes.

Clinically: The majority of HPeV infections are asymptomatic. However, the viruses are also capable of causing significant illness in the form of gastroenteritis, respiratory tract infection, and central nervous system disease. HPeV 3 in particular is a recognised cause of aseptic meningitis, and encephalomyelitis, with a particular predilection for neonates.

Diagnosis: HPeV can be found in the respiratory secretions, stool, and CSF of infected individuals. The viruses may be cultured from faecal samples, but molecular testing (PCR) is the preferred diagnostic approach.

NVRL Tests: HPeV PCR is available in the NVRL on CSFs and other relevant sample types:

Parvovirus B19 (Primate erythroparvovirus 1)

Introduction: Parvovirus B19 (B19V) is a single-stranded DNA virus from the erythroparvovirus genus of the Parvovirinae subfamily of the Parvoviridae. There are three (1-3) B19V genotypes (of little clinical significance) but just a single serotype. Humans are the natural host for B19V and the virus is distributed globally. Annual incidence typically peaks in the spring, but longer epidemic patterns are also evident with larger outbreaks every 4-5 years. Transmission occurs via droplet spread from the respiratory tract. Parvovirus infection in dogs cannot be transmitted to humans.

Clinically: B19V infection is classically associated with erythema infectiosum ('slapped cheek' or 'fifth' disease), transient aplastic crisis, arthropathy, and hydrops foetalis. However, some 50% of infections are asymptomatic. The virus causes anaemia because the cellular receptor for B19V is primarily located on red blood cells (in addition to endothelial cells, and foetal myocardium). In utero, the foetal anaemia can lead to cardiac failure and peripheral oedema ('hydrops'): the risk of foetal infection increases with gestational age, but the risk of symptomatic infection is greatest in the first trimester. B19V may also cause non-hydrops intrauterine death, probably as a result of myocarditis and cardiac dysrhythmia. Chronic B19 infection may occur in those (immunosuppressed) individuals who are not capable of producing antibodies to the virus.

Diagnosis: B19V is detectable in the blood and respiratory secretions of infected individuals. However, serology (IgM) is the diagnostic method of choice in the immunocompetent. In the immunosuppressed patient-group, B19V DNA detection in blood is the test of choice. Of note, B19V DNA may remain detectable at a low level for months following primary infection. In pregnancy, if in utero infection is suspected, B19V DNA testing can be performed on amniotic fluid or foetal blood.

NVRL Tests: B19V serology and DNA testing are provided in the NVRL. Due to the fact that < 50% of primary Parvovirus infections are asymptomatic, B19V IgG and IgM are always performed in tandem, in keeping with best practice.

Pneumocystis jirovecii

Introduction: *Pneumocystis jirovecii* (PJP) is a yeast-like fungus of the genus Pneumocystis and is one of several organisms known to cause life-threatening opportunistic infections in immunocompromised and immunosuppressed patients.

Clinically: Symptoms include fever, non-productive cough, shortness of breath and weight loss. In a small number of cases, the fungus can invade other visceral organs, such as the liver, spleen and kidney. Although it is postulated that the principal infection route is by air, the exact mode of transmission remains unknown.

Diagnosis: PJP may be detected by PCR in lower respiratory tract secretory specimens of infected individuals.

NVRL Tests: PJP infection can be confirmed by the presence of PJP nucleic acid in bronchoalveolar lavage (BAL) and/or sputa patient specimens. Please note 600 µL of sample is required for testing.

Pathogen Specific Notes:

1: PJP testing is recommended in lower respiratory tract specimens received from symptomatic immunocompromised and immunosuppressed patients only

Polio Virus

Introduction: Poliovirus (PV) is a single-stranded RNA virus from the Enterovirus genus of the Picornaviridae family. There are 3 PV types (1-3) and in the years before the global eradication programme was introduced (in 1988), PV was responsible for the paralysing some 1000 children/day around the world. Humans are the natural host for PV and the virus was distributed globally. Since the eradication programme commenced however, there are only three countries remaining in which PV transmission has not yet been interrupted (Afghanistan, Pakistan, and Nigeria). The virus is transmitted via the faecal oral route.

Clinically: Polio classically presents as an acute flaccid paralysis. However, the majority (90%) of infections are asymptomatic, presenting problems for eradication & surveillance programmes. PV causes aseptic meningitis in about 10% and classic AFP in only about 1% of infected individuals. AFP carries an associated mortality of 5% and morbidity of 85%. Post-polio syndrome occurs in later life in long-term survivors of polio.

Diagnosis: Poliovirus is shed in the stool of infected individuals, and may also be detected in the CSF of patients with aseptic meningitis or AFP. However, virus culture remains the diagnostic method of choice as molecular methods for PV can lack sensitivity.

NVRL Tests: The NVRL is a World Health Organisation (WHO) accredited Poliovirus testing Laboratory. Cell culture is the screening test of choice. For patients with clinical AFP, two stool samples, taken 24 hours apart should be sent for PV screening. Of note, PV has been eradicated in Ireland, but imported infection and subsequent transmission may occur.

Powassan Virus

Introduction: Powassan virus (POWV) is a single-stranded RNA virus that belongs to the genus *Flavivirus*. It is related to West Nile, St. Louis encephalitis, and Tick-borne encephalitis viruses. Humans become infected with POWV from the bite of an infected tick, but do not develop high enough concentrations of POWV in their bloodstreams to infect feeding ticks, and are therefore "dead-end" hosts of the virus.

POWV is maintained in a cycle between ticks and small-to-medium-sized rodents. In North America, three main enzootic cycles occur: *Ixodes cookei* and woodchucks, *Ixodes marxi* and squirrels, and *Ixodes scapularis* and white-footed mice. *Ixodes cookei* and *Ixodes marxi* rarely bite humans. *Ixodes scapularis* often bite humans and is the primary vector of Lyme disease. There are two POWV lineages in the United States (lineage 2 POWV is sometimes called Deer tick virus): both lineages have been linked to human disease. Approximately 50 cases of POW virus disease were reported in the United States over the past 10 years. Most cases have occurred in the Northeast and Great Lakes region.

Clinically: Initial symptoms of POWV disease cases include fever, headache, vomiting, and generalized weakness. The disease usually progresses to meningoencephalitis, which may include meningeal signs, altered mental status, seizures, aphasia, paresis, movement disorders, or cranial nerve palsies. Although many individuals with POWV are asymptomatic, the infection can be serious, and carries a mortality rate of 10-13%.

Diagnosis: Serology (IgM in blood or CSF) is the diagnostic method of choice. Viral RNA is detectable in blood (by PCR) but only if performed early in the course of illness.

NVRL Tests: There is currently no test for POWV available in the NVRL. Samples from patients with the appropriate clinical presentation and travel history are referred to the Rare & Imported Pathogens Laboratory in the UK.

Prions

Introduction: Prions are defined as a small infectious pathogens containing protein that are resistant to procedures that modify or hydrolyse nucleic acid. They are abnormal isoforms of a normal host-encoded protein (product of the PRNP gene) that have the capacity to induce conformational change in the normal protein when introduced into a new host. In essence, prion diseases seem to be diseases of protein conformation, as there are no differences between the amino acid sequences of the normal and abnormal protein forms. Humans are the natural host for human prion diseases, and the illness can be found worldwide. Transmission of prions may occur iatrogenically (from surgical instruments); by ingestion, or prion disease may be inherited.

Clinically: Human prion diseases or transmissible spongiform encephalopathies (TSEs) include Creutzfeldt-Jakob disease (CJD), Bovine spongiform encephalopathy (BSE), Scrapie, Kuru, Gerstmann-Straussler-Scheinker (GSS) syndrome and familial fatal insomnia (FFI). Infections are progressive and fatal with manifestations confined largely to the central nervous system. CJD exists in classic, iatrogenic, and variant forms. Variant CJD has been linked to the consumption of food contaminated with the agent of BSE.

Diagnosis: Diagnosis of prion infection is based on clinical findings in conjunction with neuroradiology and EEG results. Certain specific CSF proteins have been noted to have an association with CJD e.g. protein 14-3-3 and tau protein. Protein 14-3-3 is a marker of neuronal death. PRNP codon analysis may also be performed.

NVRL Tests: Testing for prion diseases is not performed in the NVRL.

Rabies Virus

Introduction: Rabies is a single-stranded RNA virus from the Lyssavirus genus of the Rhabdoviridae family. The virus is classified into 7 genotypes, with genotype 1 responsible for classical rabies. Rabies is enzootic in terrestrial mammals and bats in most of the world, and infects domestic animals in many parts of the developing world. Humans are typically infected through saliva from the bite of an infected animal, but exposure to bats or bat droppings (guano) is also a recognised risk.

Clinically: Rabies virus causes aggressive progressive encephalitis that may present in two forms: furious or paralytic/dumb. Rabies can often be a challenging diagnosis to make due to the length of the incubation period, which ranges from 3-12 weeks, but can be longer, and depends on the distance of the bite/inoculation from the central nervous system.

Diagnosis: Rabies virus can be detected in the CSF and saliva of infected individuals by PCR. However, direct immunofluorescent antibody staining of a skin biopsy sample, obtained from the nape of the neck (above the hairline) remains a standard diagnostic test for human rabies. Serology can also be performed on blood and CSF, and is useful for individuals who have received no prior rabies vaccine or post-exposure prophylaxis.

NVRL Tests: There are no rabies tests currently available in the NVRL: samples are referred to the Rare & Imported Pathogens Laboratory in the UK.

Pathogen-Specific Notes:

1: If suspecting rabies virus infection, please contact a member of the NVRL clinical team to ensure that transport of samples to the UK can be expedited: specimens received without prior communication will ***not*** be routinely tested

Respiratory Syncytial Virus (Human orthopneumovirus)

Introduction: Respiratory Syncytial Virus (RSV) is a single-stranded RNA virus from the Pneumovirus genus of the Paramyxoviridae family. There are two RSV types (A&B), and the virus constitutes the major respiratory pathogen of young children, with practically all children infected in the first three years of life. Humans are the natural host for RSV, and the virus is distributed globally, with seasonal outbreaks each year that typically peak between January and March in Ireland. Transmission occurs via large particle aerosols and fomites.

Clinically: RSV classically causes bronchiolitis in children, but can also cause upper and lower respiratory tract infections (which may be severe) in immunocompromised individuals (especially transplant recipients).

Diagnosis: RSV is readily detectable (IF or PCR) in the respiratory secretions of infected individuals. .

NVRL Tests: PCR is the routine test and IF is available only on request.

Rhinovirus

Introduction: Human Rhinovirus (HRV) is a single-stranded RNA virus from the Picornaviridae family. There are more than 100 different HRV serotypes, divided into three different groups (A-C). Historically, groups A&B were also known as Major & Minor, and viruses from both of these groups grew in cell culture. HRV-C however does not grow readily in culture and was a discovery of the molecular era of virology. Humans are the natural host for HRV and the viruses are distributed globally. Transmission occurs via aerosols and fomites.

Clinically: HRV is the major cause of the common cold, and was traditionally associated only with infections of the upper respiratory tract because the virus grew optimally at a temperature of 33-35 degrees C. However, it is being increasingly recognised that HRV is a significant cause of LRTI (and hospitalisation), especially in young children. In addition, HRV may be a frequent cause of exacerbations of asthma and COPD.

Diagnosis: HRV is be detected by PCR in the respiratory secretions of infected individuals.

NVRL Tests: PCR is the test of choice for HRV at NVRL. HRV culture is available.

Rift Valley Fever Virus

Introduction: Rift Valley fever virus (RVFV) is a single-stranded RNA virus from the Phlebovirus genus of the Bunyaviridae family, named for the Rift Valley in Kenya where it was first discovered. Rift valley fever itself is a disease of the domestic ruminants (sheep, cattle & goats) that, in conjunction with its mosquito vector, form the natural reservoir for the virus. RVFV is found in mainland Africa, and although the virus is mosquito-borne, most human infections are acquired from infected animal tissue. Human to human transmission, though theoretically possible (by aerosol or intranasal inoculation), has not been reported.

Clinically: RVFV human infection manifests as a moderate-severe febrile illness in the majority. However, focal retinal ischaemia (1-20%), encephalitis (<1%) and haemorrhagic fever (<1%) can also occur.

Diagnosis: Serology is the mainstay of diagnosis, although viral RNA may be detected in blood by PCR in the first week of illness.

NVRL Tests: There are no tests available for RVFV in the NVRL. Specimens from individuals with the appropriate presentation and travel history are referred to the Rare & Imported Pathogens Laboratory in the UK.

Ross River Virus

Introduction: Ross River Virus (RRV) is a single-stranded RNA virus from the Alphavirus genus of the Togaviridae family, and is the most common human arthropod-borne viral infection in Australia. Although the virus was named for its country of origin, RRV is also found in Papua New Guinea, Fiji, and the Cook Islands. Marsupials and rodents are believed to provide the virus' natural reservoir, which is transmitted by mosquito. Human to human transmission does not occur.

Clinically: RRV infections are asymptomatic in the majority (>95%) of individuals, but the classic presentation comprises a triad of arthralgia, rash, and fever, leading to its alternative name of 'epidemic polyarthritis'. Although RRV is typically not fatal, it is a cause of significant morbidity with symptoms lasting for 6-9 months.

Diagnosis: In the context of the appropriate travel history, RRV infection may be diagnosed clinically. If laboratory confirmation is required, viral RNA is detectable in blood early in the course of infection, with IgM the mainstay of diagnosis thereafter. Of note, IgM may persist for 2 years.

NVRL Tests: There are no tests available for RRV in the NVRL at present. Specimens from individuals with the appropriate clinical presentation and travel history are referred to the Rare & Imported Pathogens Laboratory in the UK.

Rotavirus

Introduction: Rotavirus is a double-stranded RNA virus from the Reoviridae family that takes its name from the Latin for wheel ('rota') and its appearance under the electron microscope. There are 10 groups of rotaviruses (designated A-J) with multiple types within each group. Human rotavirus infections are primarily caused by Group A, within which the different subtypes are distinguished on the basis of their 'G' and 'P' types: in a manner similar to influenza virus, and to date there are 36 G types and 51 P types. Most human rotaviruses currently circulating belong to types G1-4, 9 or 12, and P4, [6] or [8]. Humans are the natural hosts for group A rotaviruses and the viruses are distributed globally. The virus is transmitted via the faecal-oral route and infection rates typically peak in winter and spring in temperate climates.

Clinically: Rotavirus is the major cause of gastroenteritis globally in children <5yrs of age. Typical symptoms include diarrhoea, vomiting, and dehydration. Rarely, central nervous system manifestations or chronic infection can occur. The Rotavirus vaccine, Rotarix, was introduced for all children born in Ireland from September 2016 onwards. Vaccine is given in 2 doses at 2 and 4 months of age.

Diagnosis: Rotavirus is readily detectable in the stool samples of infected individuals. PCR provides the capacity to identify the circulating virus type

NVRL Tests: Real-time PCR is the test of choice in the NVRL for the diagnosis of rotavirus infection. Rotavirus genotyping is also available. Vaccine-derived Rotavirus (Rotarix) is detectable for several weeks post vaccination and can be differentiated from wild-type virus

Rubella Virus

Introduction: Rubella virus is a single-stranded RNA virus of the Togaviridae family. There are two distinct viral clades, and 13 genotypes, but only one serotype: the different genotypes are of little clinical significance. Humans are the only known reservoir for Rubella virus and the virus is distributed globally, although it has been eradicated through immunisation programmes in some countries. The virus is transmitted by respiratory droplet, but is not terribly efficient, with close and prolonged contact typically required for the acquisition of infection.

Clinically: Rubella virus is probably best known for the congenital rubella syndrome (CRS) that affects the heart, eyes, and ears. Infection in the first 10 weeks of pregnancy confers the greatest risk to the foetus, with defects rare if infection occurs after 20 weeks. CRS was the primary reason for the introduction of universal childhood immunisation against rubella. In the non-partum setting, rubella classically presents as a maculopapular rash – accompanied by lymphadenopathy – starting at the face and spreading to the trunk and the limbs. Up to 70% of post-pubertal females develop an arthropathy with primary infection, and 20-50% of infections are subclinical.

Diagnosis: Rubella virus RNA is present in oral fluid samples from infected individuals and in infected neonates can also be detected in urine, blood, respiratory secretions, and CSF. Oral fluid is the specimen of choice for children (as it's non-invasive).

NVRL Tests: The NVRL is the WHO designated Rubella reference laboratory for Ireland. As such, the full range of testing options is available: serology and molecular testing (PCR) are routinely performed, and virus culture is available on request. Acute Rubella infections continue to be rare in Ireland, one case was reported in Feb 2020 (the last reported case was in 2009)

Pathogen-Specific Notes:

1: A rubella IgG titre of >10IU/ml is considered protective against primary infection. Individuals with an antibody level of 5-10IU/ml will be reported by the NVRL as Rubella IgG antibody detected at low level. If this person has documented evidence of receiving at least one dose of Rubella vaccine no further vaccination is necessary.

Saint Louis Encephalitis Virus

Introduction: Saint Louis Encephalitis Virus (SLEV) is a single-stranded RNA virus from the Flaviviridae family. There are 7 genetic lineages of the virus, but these are of more use epidemiologically than clinically. Prior to the introduction of West Nile Virus in 1999, SLEV was the major cause of epidemic encephalitis in the US. Sparrows and mosquitoes are the virus' natural hosts, and it is widely distributed from southern Canada to South America. Humans are infected by mosquitoes: human to human transmission does not occur.

Clinically: Although the majority (>90%) of human SLEV infections are asymptomatic, infection of the central nervous system (CNS) as the name suggests, ranges from aseptic meningitis to encephalitis, and carries a mortality rate of 10-20%. CNS disease is more common in the elderly.

Diagnosis: Detection of IgM in blood is the diagnostic method of choice. Viral RNA is detectable in blood by PCR, but only in the early/pre-clinical stage of the disease, so unless SLEV is suspected very early, PCR is unlikely to be positive.

NVRL Tests: SLEV is not tested at the NVRL. Specimens are therefore referred to the Rare & Imported Pathogens Laboratory in the UK for testing.

Severe Acute Respiratory Syndrome Coronavirus (SARS CoV)

Introduction: Severe acute respiratory syndrome Coronavirus (SARS-CoV) is a single stranded RNA virus from the Coronaviridae family. The virus first appeared in China in November 2002 and in February 2003 spread globally to cause the first pandemic of the 21st century. The virus made the jump from bats to humans via an intermediate host that was most likely the palm civet in animal markets in China. Fortunately, the viral load peaked late in the course of the illness which meant that good infection control with quarantining of those infected and exposed quickly controlled the pandemic. In the early stages however, the majority of those infected were healthcare workers. Over 8000 people were infected worldwide with a mortality rate of about 10%. SARS-CoV has not reappeared since 2004.

Clinically: As the name suggests, SARS causes a severe ARDS-like respiratory syndrome, with lymphopaenia and progressive renal impairment also documented.

Diagnosis: SARS-CoV is present in the respiratory secretions of infected individuals and is readily detected by PCR.

NVRL Tests: Requests for SARS-CoV are referred to the UK for diagnosis.

Severe Acute Respiratory Syndrome Coronavirus Type 2 (SARS-CoV-2)

Introduction: SARS-CoV-2 is a single stranded RNA Novel Coronavirus from the *Sarbecovirus* subgenus of the *Coronaviridae* family, which was first identified in China in December 2019, following an outbreak of an acute respiratory disease in Wuhan province. This virus rapidly spread across the world leading to a global pandemic with significant mortality. The possible animal source of this new virus is as yet undetermined, but believed to be bats.
In Quarter 1 of 2020 cases of infection with this Novel Coronavirus were identified in Ireland. Subsequently the virus was named as SARS-CoV-2 with the resulting disease termed COVID-19. A global COVID -19 Pandemic was declared by WHO in March 2020.

Clinically: SARS-CoV-2 is a Category 3 Respiratory pathogen, highly infectious, spread through human-to human transmission and capable of illness which can range from mild to severe, and in some cases, can be fatal. The following symptoms may develop in the 14 days after exposure to someone who has COVID-19 infection:

- Cough
- Shortness of breath or difficulty in breathing
- Fever (38.0°C [100.4°F] or greater)

Other symptoms that are less common and may affect some patients include aches and pains, nasal congestion, headache, conjunctivitis, sore throat, diarrhoea, loss of taste or smell or a rash on skin or discoloration of fingers or toes. These symptoms are usually mild and begin gradually. It is important to note some people infected with the virus can be asymptomatic. One in five patients will become seriously ill. Generally, SARS CoV-2 infection causes more severe disease in people with weakened immune systems, older people and those with long-term medical problems such as high blood pressure, diabetes, cancer and chronic lung disease.

Diagnosis: SARS-CoV-2 is present in the throat and nasal passages, bronchoalvelor lavage (BAL) and sputum of infected individuals, and is readily detected by PCR. Virus can also be detected in stool samples.

NVRL Tests: SARS-CoV-2 RNA testing is available at the NVRL using a qualitative RT-PCR. Tests also exist for SARS-CoV-2 antibody although these are not currently recommended for routine diagnosis of current or prior infection – these tests are carried out on request. Whole Genome Sequencing (WGS) is carried out at NVRL for tracking Variants of Concern, and monitoring outbreaks.

Sindbis Virus

Introduction: Sindbis virus is a single-stranded RNA virus from the Alphavirus genus of the Togaviridae family, named for the place in Egypt where it was first detected. In fact, Sindbis virus is probably the most widely distributed (throughout the world) of all the known arthropod-borne viruses, but it is one of the least virulent. The virus is maintained principally in birds, and transmitted by mosquitoes. Human to human transmission does not occur.

Clinically: Despite its global distribution, clinically apparent disease with Sindbis virus is uncommon. The majority of infections are asymptomatic, and the classic presentation is one of rash & arthralgia. Sindbis virus is responsible for Ockelbo disease (Sweden), Pogosta disease (Finland), and Karelian Fever (Russia).

Diagnosis: Serology (IgM) is the diagnostic method of choice, as the viraemia associated with Sindbis virus is low level & transient. Of note, IgM may remain detectable for 3-4 years following a primary infection.

NVRL Tests: There is currently no test for Sindbis virus available in the NVRL. Samples from patients with the appropriate travel history and clinical presentation are referred to the Rare & Imported Pathogens Laboratory in the UK.

Smallpox Virus

Introduction: Smallpox virus is a double-stranded DNA virus from the Orthopox genus of the Poxviridae family. There were two different strains (major & minor), one of which carried an associated mortality of 30% and the other 1%. The disease & virus were eradicated in 1977 following a successful immunisation programme coordinated by the world health organisation (WHO). Humans were the natural reservoir for smallpox and the virus was distributed globally. Person to person transmission occurred via the airborne route, or infective droplets.

Clinically: The classic centrifugal smallpox rash was vesiculo/pustular in appearance, but could also manifest as flat-type, or haemorrhagic. In addition, atypical rashes were described in vaccinated individuals. The cause of death was probably the result of renal/respiratory failure and septic shock, though this has not been definitively clarified.

Diagnosis: The diagnosis of smallpox was historically clinical. However, the virus was also readily detectable in skin lesions by EM or PCR.

NVRL Tests: Smallpox has been officially eradicated, so its re-emergence would probably signify an intentional act of bioterrorism (through the release of virus from severe storage facilities), and constitute a national & international health emergency due to the lack of immunity in the general population. EM facilities are however available in the NVRL should the need arise.

Tick Borne Encephalitis Virus

Introduction: Tick borne encephalitis virus (TBEV) is a single-stranded RNA virus from the Flaviviridae family. However, the name is slightly misleading in that there is no single TBEV. The name refers to 3 viral subtypes (central or western European; Russian spring-summer virus or Far Eastern; and Siberian or Vasilchenko) that in turn constitute one subgroup of 6 (along with Louping ill [UK]; Omsk haemorrhagic fever virus [Siberia]; Langat [Malaysia]; Kyasanur Forest [India]; and Powassan [Ontario]) within 1 of 3 mammalian major groups (along with Seabird & Kadam) of tick-borne flavivirus.

The three main TBEV subtypes cause around 3000 cases of encephalitis in Europe each year, but the incidence is increasing due to climate change and more widespread vector distribution. The natural hosts of TBEV are small rodents and ticks, but goat and deer are also required to maintain the tick population. As mentioned above, the viruses are endemic across a large swathe of mainland Europe (from central Europe as far as China). Humans become infected by tick bite or through the ingestion of infected milk from sheep & goats. Human to human transmission does not occur.

Clinically: The central European variant of TBEV presents as a classic biphasic illness, with an influenza-like illness followed by a brief remission, then progression to meningitis/encephalitis. Mortality with CE TBEV ranges from 1-5%. Conversely, Russian Spring Summer virus presents as a more aggressive monophasic illness (fever – meningitis – encephalitis/coma – RIP) with a significantly higher mortality rate of 10-35%.

Diagnosis: Serology (IgM in blood) is the diagnostic method of choice. Viral RNA is detectable in blood (by PCR) but only if performed early in the course of illness. Of note, IgM may persist for 10-12 months following primary infection.

NVRL Tests: Serological investigations of TBE IgM/IgG are available as part of the Flavivirus Euroimmun screen for samples from patients with the appropriate clinical presentation and travel history. Positive samples are referred to the Rare & Imported Pathogens Laboratory in the UK for confirmation.

Torovirus

Introduction: Torovirus is a single-stranded RNA virus from the Torovirus genus of the Coronaviridae family. Although the virus was initially isolated from the stool of individuals with gastroenteritis, a definitive disease association has not yet been proven. To date, only a single human Torovirus genotype has been described and is distributed worldwide. Humans are believed to be the natural host and transmission is likely to occur via the faeco-oral route.

Clinically: Torovirus has been associated with diarrhoeal illness, both acute & chronic. The significance of Torovirus in human gastroenteritis remains to be elucidated, though it has been detected in up to 25% of symptomatic children.

Diagnosis: Torovirus is readily demonstrable in stool by EM, EIA, or PCR.

NVRL Tests: There is currently no test for Torovirus available in the NVRL.

Torque Teno Virus

Introduction: Torque Teno Virus (TTV) is a single-stranded DNA virus from the Anellovirus genus of the Circoviridae family. The virus was first discovered in 1997 from a patient with non-A-E hepatitis. Despite this initial presentation however, no human pathogenicity for TTV has yet been established. Nevertheless, multiple TTV genotypes have been identified and TTV DNA can be retrieved from >90% of adults worldwide. In addition, TTV viral loads have been reported to be higher in some immunocompromised patient cohorts. Humans are presumed to be the natural hosts for TTV, although TTV-related viruses have also been found in chimpanzees and monkeys. The mode of transmission is not known, although higher viral loads in nasal secretions compared with blood may suggest a possible respiratory route. Further work is required to ascertain the significance of this highly diverse group of viruses.

Clinically: No definitive disease association has been identified for TTV.

Diagnosis: TTV can be detected in infected individuals by PCR.

NVRL Tests: There is currently no test for TTV available in the NVRL.

Toscana Virus

Introduction: Toscana virus (TOSV) is a single-stranded RNA virus from the Phlebovirus genus of the Bunyaviridae family. Whilst the virus was originally discovered in Italy (hence the name) it has since been reported in France, Portugal, Spain, Turkey, Croatia, Greece, Cyprus & Tunisia, and constitutes a major cause of meningitis and encephalitis each summer. The natural reservoir (probably rodents &/or bats) for TOSV has not yet been identified, but it is possible that virus' vector – the phlebotomine sandfly – also functions as a virus reservoir. Should this turn out to be the case, TOSV could become more widespread (depending on the habitat of the natural reservoir host) as the sandfly vector is widely distributed across Europe. Human to human transmission does not occur.

Other Phleboviruses circulating in Europe include Sandfly fever Naples virus (SFNV), Sicilian virus (SFSV), and Cyprus virus (SFCV). In contrast to TOSV, these viruses do not typically cause CNS disease. They classically present with 'three-day' or 'pappataci' fever, a self-limiting febrile illness associated with retro-orbital pain, myalgia, and malaise. Pappataci derives from the Italian word for sandfly.

Clinically: TOSV infections classically present as meningitis. However, some infections are asymptomatic, some result in a febrile illness, and around 10% of individuals will develop encephalitis. Skin lesions (due to sandfly bites) should also be present.

Diagnosis: Serology is the diagnostic approach of choice for TOSV infection, although viral RNA may be detected in blood early in the course of illness. TOSV RNA may also be detected in CSF in cases of central nervous system (CNS) disease.

NVRL Tests: There is currently no test for TOSV in the NVRL. Specimens from patients with the appropriate clinical presentation and travel history are referred to the Rare & Imported Pathogens Laboratory in the UK.

Toxoplasma gondii

Introduction: *Toxoplasma gondii* is a protozoan and obligate intracellular parasite that exists in several infectious forms: tachyzoite, tissue cysts (containing bradyzoites), and oocysts (containing sporozoites). The tissue cyst form is transmitted through undercooked or raw meat, and the oocyst (present in the small intestine of cats & other members of the feline family) is responsible for transmission through soil, water, or soil contaminated with infected cat faeces. *T gondii* is distributed worldwide and infects most species of warm-blooded animals. Humans usually become infected by consumption of raw or undercooked meat (that contains cysts) or by accidental ingestion of oocysts from contaminated soil, food, or water. Members of the feline family are definitive hosts. Intermediate hosts (sheep, pigs, cattle) can have tissue cysts in the brain, myocardium, skeletal muscle and other organs. These cysts remain viable for the lifetime of the host.

Clinically: Up to 50% of acutely infected people do not have identifiable risk factors or symptoms. In symptomatic individuals, clinical findings may include lymphadenopathy (frequently cervical) and/or an infectious mononucleosis-like syndrome. Rarely, severe disseminated infection (myocarditis/pneumonitis/hepatitis/encephalitis) may occur. In chronically infected immunocompromised patients, *T gondii* reactivation can result in life-threatening encephalitis, pneumonitis, or disseminated disease. *T gondii* is also associated with congenital infection, with the risk of severe congenital disease highest in the 1st and 2nd trimesters. However, the risk of transmission to the foetus is greatest in the 3rd trimester. Of note, <90% of congenitally infected infants may be asymptomatic at birth, but will still go on to develop sequelae (visual/hearing impairment, learning disability, mental retardation) in the first months-years of life.

Diagnosis: Serologic tests are the primary means of diagnosing primary and latent infection, with molecular testing (PCR) of body fluids or histological staining of biopsy tissue reserved for confirmation of Toxoplasmosis. Molecular testing is often used to confirm a diagnosis of *in utero* Toxoplasma infection. IgM & IgA testing should be performed in neonates suspected of congenital infection.

NVRL Tests: T gondii specific IgM, IgA, IgG, and IgG avidity assays are available in the NVRL. Specimens for Toxoplasma PCR are sent to the Toxoplasma Reference Laboratory in Swansea.

Treponema Pallidum

Introduction: *Treponema pallidum* (TP) is a spirochaete, and the causative agent of Syphilis. The infection is found worldwide, with transmission due to direct contact with an infectious lesion (typically during sexual contact), or vertically (from mother to child). There has been a significant resurgence in TP infection in recent decades, with increased rates of infection in men who have sex with men (MSM) and HIV-infected individuals.

Clinically: Acquired syphilis is divided into three main stages: primary, secondary, and tertiary. Latent syphilis defines a period between the secondary & tertiary stages when individuals remain infected (serologically positive) but asymptomatic. Latent syphilis is further divided into early & late categories, which in simple terms reflect infectivity of the patient. Signs and symptoms of TP infection vary depending on the stage of infection, with primary & secondary stages of infection being self-limiting, and resolving spontaneously without treatment. Left untreated however, patients remain at risk of tertiary infection (involving cardiovascular system, or CNS infection) that can present 15-30 years following initial infection. Further information in relation to the management of *T pallidum* infection is available from the BASHH website. Congenital infection may present with early or late manifestations. Untreated asymptomatic infants may develop sequelae of TP infection many years after birth. For further information please refer to National Guidelines. Syphilis IgM is not tested on infants. Please refer to the following document: 'Preventing Perinatal Transmission Guidelines' on the ssstdi.ie website (Society for the Study of STDs in Ireland). *See below of sample volume requirements.

Diagnosis: Serology remains the mainstay of diagnosis for TP infection, with the diagnostic assays divided into two distinct groups: non treponemal-specific tests, e.g. RPR; and specific treponemal tests, e.g. treponemal enzyme immunoassay (EIA)/TPHA to detect IgG and/or IgM. Of note, serology may be negative in early primary infection with insufficient time lapsed to develop an antibody response: therefore, if clinical suspicion is high, a follow up serum sample should be collected in these cases. Diagnosis of TP infection can also be made by identifying spirochaetes with dark ground microscopy or by molecular techniques (PCR) if ulcers/chancres are present at the time of presentation.

NVRL Tests: Serum samples are screened using the Abbott Architect *T pallidum* total antibody (IgG/IgM) assay. Samples yielding positive results on the Architect are confirmed by further treponemal and non-treponemal TPHA/EIA/immunoblot tests. In newly diagnosed cases, a specific *T. pallidum* IgM assay is also performed. Investigation of congenital infection is performed in accordance with National Guidelines that can be found on the NVRL website. Dark ground microscopy & PCR for *T pallidum* infection are not available in the NVRL.

NB *The volume of blood/serum required for testing in relation to diagnosis of congenital infection is a minimum of 1.25 ml blood/250µl serum

Trichomonas vaginalis

Introduction: *Trichomonas vaginalis* (TV) is a flagellated protozoon. The organisms has 4 anterior flagella which give its characteristic twisting and wriggling movement. In women the organism is found in the vagina, urethra and paraurethral glands. Urethral infection is present in 90% of infected women, although the urethra is rarely the the sole site of infection. In men infection is usually of the urethra, although trichomonads have been isolated from the subpreputial sac and lesions of the penis. Once the organism is in contact with the epithelium it changes form into an amoeboid-like structure and spreads along the epithelium which facilitates host receptor binding. Transmission of TV is almost exclusively through sexual intercourse as, due to its site specificity, infection only occurs following intravaginal or intraurethral inoculation of the organism. TV is considered the most prevalent non-viral sexually transmitted disease in the world and the WHO estimates that greater than 250 million individuals are infected each year. However, there are vast differences in the reported incidence between continents, with Northern Europe among countries with the lowest estimated TV prevalence.

Clinically: In women, an estimated 10–50% of TV infections are asymptomatic. The most common symptoms are non-specific and include vaginal discharge, vulval itching and dysuria. Occasionally, woman may present with low abdominal discomfort or vulval ulceration.
In men, as many as 15 to 50% of TV infections are asymptomatic; as a result, men usually only present for testing following a TV diagnosis in a female sexual partner. However, non-specific symptoms including urethral discharge, dysuria, urethral irritation and urinary frequency may occur following infection.

Diagnosis: Testing for TV is recommended in women evidence of vaginal discharge or vulvitis, Testing in men is recommended for TV contacts, and should be considered in those with persistent urethritis. Nucleic Acid Amplification tests (NAATs) are the preferred investigation for diagnosis of TV infection.

NVRL Test: TV testing is performed in the NVRL using the Aptima TV assay (Hologic). Specimen collection advice is included in the APTIMA kits provided by the NVRL. Two types of specimen collection device (SCD) are available: Multitest swab and urine. Following specimen collection, the SCD are stable at room temperature for 60 days.

Pathogen-Specific Notes:

1: Only specimens collected in APTIMA collection devices can be tested in the NVRL.

2: Please refer to section 5.5 Specimen Collection for information related to the APTIMA collection devices. Please pay particular attention to the sample level required in the SCD.

Varicella Zoster Virus

Introduction: Varicella Zoster Virus (VZV) is a double-stranded DNA virus from the Alphaherpesvirinae subfamily of the Herpesviridae family. There are 5 proposed clades (1-5) of VZV that demonstrate a distinct geographic variation, but as of yet no clinical significance has been attributed to specific clades. Humans are the natural host for VZV and the virus is distributed worldwide. Transmission occurs via the airborne route (from skin lesions) and via respiratory secretions. Primary infection with VZV typically peaks in the Spring in temperate climates.

Clinically: VZV is practically unique in virological terms in that it causes distinct primary (chickenpox) and secondary (zoster) clinical syndromes. Following primary infection, the virus remains latent for life in the dorsal root ganglia of the spinal nerves, reactivating typically later in life as T cell function wanes. Reactivation may occur more frequently and at a younger age in the immunocompromised. Primary VZV infection is typically self-limiting in childhood, but pneumonitis and meningoencephalitis may complicate infection. Reactivation of VZV may also be complicated by severe post-herpetic neuralgia. Varicella zoster virus is also capable of causing congenital infection. The risk to the foetus is greatest (albeit still low at 2%) in weeks 13-20 gestation. Neonatal infection can also occur if the non-immune mother develops primary infection within 7 days of delivery.

Diagnosis: VZV DNA is readily detectable in vesicular fluid and scrapings of the skin lesions: in addition, DNA may be detected in respiratory secretions. Serological testing is of more utility in determining prior infection (IgG) but IgM testing can be performed if swabs of the lesions are not available.

NVRL Tests: VZV serology and molecular testing, are available in the NVRL.

Pathogen-Specific Notes:

1: VZV IgM testing is ***not*** the test of choice for the diagnosis of Primary infection, as a negative IgM result does not exclude a diagnosis of chickenpox.

2: VZV IgM testing is ***not*** recommended for the diagnosis of ‘shingles’ (Herpes Zoster)

Venezuelan Equine Encephalitis Virus

Introduction: Venezuelan Equine Encephalitis Virus (VEEV) is a single-stranded RNA virus from the Alphavirus genus of the Togaviridae family. There are 6 VEEV subtypes (A-F) but they are of little clinical significance. VEEV – as the name suggests – is an important cause of central nervous system (CNS) disease in central & South America, where the virus is widely distributed. Rodents and birds are the natural hosts for the mosquito-transmitted VEEV. Human to human transmission does not occur, although the virus has been isolated from the pharynx of infected individuals, and airborne infection has been reported in a number of laboratory outbreaks of VEEV.

Clinically: VEEV infection ranges from asymptomatic (≈ 40%), to a self-limiting febrile illness (15-20%), and encephalitis. CNS involvement of some description occurs in 40-50% (severe in 5-15%) of infected individuals (more commonly in children), and carries a mortality rate of 10-25%.

Diagnosis: Detection of IgM in blood is the diagnostic method of choice for VEEV. Viral RNA is present in blood early in the course of infection, and can be readily detected – if suspected – by PCR.

NVRL Tests: VEEV is not tested at the NVRL. Specimens are therefore referred to the Rare & Imported Pathogens Laboratory in the UK for testing.

Viral Haemorrhagic Fevers

Introduction: The term viral haemorrhagic fever in fact refers to a number of different viruses from distinct virus families. As such, in this user manual, each 'haemorrhagic fever' virus has been afforded its own section. The relevant viruses to which the term VHF refers are:

Virus Name	**Virus Family**
Lassa	Arenaviridae (see Lymphocytic Choriomeningitis)
Ebola	Filoviridae
Marburg	Filoviridae
Crimean Congo Haemorrhagic Fever	Bunyaviridae

Please also refer to the Report of the Scientific Advisory Committee of the Health Protection Surveillance Centre "The Management of Viral Haemorrhagic Fevers in Ireland", published November 2012, for further guidance.

Pathogen-Specific Notes: Viral Haemorrhagic fever (VHF) testing is only done by prior arrangement with clinical team and as per guidelines at:
www.hpsc.ie/hpsc/A-Z/Vectorborne/ViralHaemorrhagicFever/Guidance/
The NVRL must be notified before dispatch from hospital of any suspect VHF specimens. Please contact the laboratory on 01-7161321/7164440.

Western Equine Encephalitis Virus

Introduction: Western equine encephalitis virus (WEEV) is a single-stranded RNA Alphavirus of the Togaviridae family, and is an important cause of encephalomyelitis in horses and humans. Compared with the similarly-named EEEV, infections with WEEV are more common but less severe. Birds are the natural hosts for WEEV, which is transmitted by mosquitoes. The virus is traditionally associated with the pacific coast and the great plains of the US (hence the name), but is also found in Canada, Central, and South America.

Clinically: The majority of infections with WEEV are asymptomatic, but patients may also experience a simple febrile illness. Encephalitis – the classic presentation – occasionally occurs in adults, but is more common in children. When encephalitis does occur, mortality rates can be as high as 10%.

Diagnosis: Detection of IgM is the test of choice for diagnosing WEEV infection. Although viral RNA is detectable in blood early in the course of illness, PCR may be negative by the time the diagnosis considered.

NVRL Tests: WEEV is not tested at the NVRL. Specimens are therefore referred to the Rare & Imported Pathogens Laboratory in the UK for testing.

West Nile Virus

Introduction: West Nile Virus (WNV) is a single-stranded RNA Flavivirus that was discovered in the West Nile region of Uganda in 1937. Historically, WNV was enzootic throughout Africa, parts of Europe, Asia, and Australia. However, the virus received little attention until 1999 when it was introduced into the US, and managed to spread across the entire continent within a few short years. The virus is enzootic in birds, transmitted by mosquitoes, and causes disease primarily in humans and horses. Human to human transmission does not typically occur, but WNV has been transmitted to recipients of blood transfusions and transplanted organs.

Clinically: The majority (60-80%) of WNV infections are asymptomatic. Twenty to 40% of those infected will experience west Nile fever (an unpleasant influenza like illness that may require hospitalisation). A small subset of these patients (about 1 in 150 of all WNV infections) will progress to neuroinvasive WNV infection, as defined by acute flaccid paralysis, meningitis, encephalitis, and ocular manifestations. The risk of severe disease is greatest in the elderly, with a 20-fold increase in risk for neuroinvasive disease and death in those over 50 years of age.

Diagnosis: Detection of IgM is the test of choice for the diagnosis of WNV infection. Viral RNA is present in blood early in the course of the disease, but the viraemic period is short, so serology is always required.

NVRL Tests: West Nile Virus IgM and IgG testing is routinely performed on samples received at the NVRL for West Nile Virus investigations. Reactive samples at then processed on a Flavivirus Immunofluorescence Assay (IFA) panel for confirmation.

WU Polyomavirus

Introduction: WU Polyomavirus (WUPyV), named for Washington University, where it was first identified in 2007, is a double-stranded virus from the Polyomaviridae family. The virus was initially discovered in the nasopharyngeal aspirate of a three year old with pneumonia, and subsequently has been detected in respiratory secretions, stool samples, and blood. The virus is distributed globally and infection appears to occur in the majority of the human population early in the life, with seroprevalence rates approaching 60% in 10-15yr olds. The mode of transmission for the virus however has not yet been determined.

Clinically: Based on the available data, WUPyV certainly causes infection in humans (as evidenced by the seroprevalence studies), and would appear to have the capacity to cause respiratory tract infections in a small number of individuals, and. However, it is an uncommon cause of symptomatic RTI, and the virus is often found in the presence of other respiratory pathogens: in addition, WUPyV has also been detected in asymptomatic individuals. Consequently, routine screening for WUPyV is not recommended.

Diagnosis: WUPyV DNA has been detected primarily in the respiratory secretions of individuals with respiratory symptoms.

NVRL Tests: There is currently no test for WUPyV in the NVRL.

Xenotropic Murine Leukaemia Virus-like Retrovirus

Introduction: Xenotropic murine leukaemia virus-like Retrovirus (XMRV) is a single-stranded RNA virus from the gammaretrovirinae subfamily of the Retroviridae. XMRV is a relatively recently discovered retrovirus that was first identified in familial prostate cancer cell lines, and subsequently detected in a subset of prostate tumour samples. The virus attained a degree of notoriety when a group reported that it had been detected in a cohort of individuals with chronic fatigue syndrome. However, these findings were not reproducible in other populations and the original paper was ultimately retracted. As the name suggests, XMRV is similar in sequence and properties to xenotropic murine leukaemia viruses, so at present, it would appear that most reported XMRV isolates are laboratory contaminants.

Clinically: XMRV has been detected in prostate tumours, but no causative association has been identified.

Diagnosis: To date, XMRV has predominantly been detected by PCR in tissue/blood samples.

NVRL Tests: There is no test available in the NVRL for XMRV.

Yellow Fever Virus

Introduction: Yellow fever virus is a single-stranded RNA virus from the Flaviviridae family. Historically, yellow fever was the first human disease demonstrated to be caused by a filterable agent (i.e. a virus). There are at least 7 YFV genotypes and the virus is endemic in the tropics on both sides of the Atlantic. Distribution is restricted to the regions between latitudes 10N & 40S in the Americas, and 16N & 10S in Africa. The virus is transmitted by mosquitoes, and human to human transmission does not occur: however, humans can sustain YFV epidemics if the mosquito host is present.

Clinically: Yellow fever virus infection manifests as the classic biphasic illness, although the majority of infections probably abort following the initial febrile phase (associated with strawberry tongue & relative bradycardia). The second phase of the illness comprises haemorrhage, hepatic & renal disease, and carries an associated mortality of 20-50%.

Diagnosis: Detection of IgM in blood is the diagnostic method of choice. Viral RNA is also detectable early in the course of illness, but may be absent if the patient presents in the haemorrhagic phase of the illness.

NVRL Tests: Yellow fever virus is a component of the Euroimmun Flavivirus IFA screening assay performed in the NVRL. Specimens testing positive in the screening assay are forwarded to the Rare & Imported Pathogens Laboratory in the UK for confirmatory testing.

Pathogen-Specific Notes: If suspecting Yellow Fever Virus infection, it is essential to provide a comprehensive vaccine history to the diagnostic laboratory, as prior vaccination against Yellow Fever Virus may result in positive YFV serology.

Zika Virus

Introduction: Zika virus is a mosquito-borne single-stranded RNA member of the *Flaviviridae* that was first discovered in the Zika forest in Uganda in 1947. The virus reservoir appears to be monkeys and outbreaks have been reported in tropical Africa and Southeast Asia.

Clinically: The main clinical symptoms are fever, conjunctivitis, transient arthritis/arthralgia, and a maculopapular rash. In general, the disease is mild and self-limiting.

Diagnosis: The virus can be detected in the blood of infected patients by PCR early in the course of illness. From day 5 post-onset of fever, specific IgM antibodies are present.

NVRL Tests: Specimens from patients with the appropriate travel history and clinical presentation are screened in the NVRL. Serology (IgM and IgG in blood) is the diagnostic method of choice. Viral RNA is detectable in blood (by PCR) but only if performed early in the course of illness. Samples that yield a positive result on the screening assay are referred to the Rare and Imported Pathogens Laboratory in the UK for supplementary/confirmatory testing.

APPENDIX 1.0

Procedure for the Transport of Infectious Substance Category A

Guidance for Collection, Transport and Submission of Specimens for VHF testing

Category A
An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals (see WHO Guidance on regulations for the transport of infectious substances 2017-2018).
The proper shipping name for Category A, UN2814 is "INFECTIOUS SUBSTANCE, AFFECTING HUMANS" and must be packaged for carriage by road in accordance with ADR Packing Instruction P620
Examples of Category A pathogens would be the viruses associated with Viral Haemorrhagic Fever (VHF): Ebola, Marburg, Lassa, Crimean Congo Haemorrhagic Fever, and smallpox virus.

Packaging and transport of biological specimens within a facility
Viruses causing haemorrhagic fevers are classified as **Category A** infectious pathogens.
Within the hospital, specimens should be transported according to local arrangements for high-risk samples.
Precautions should include:

- primary containers must be leak-proof and a waterproof, leak-proof seal must be used
- secondary containers should be placed in a good quality box, which is well taped up and clearly labelled "Pathological Specimen - Open only in Laboratory";
- specimens should be transported by hand by a responsible person using the above packaging. Vacuum-tube systems **must not be used** for transportation of specimens within hospitals or laboratories
- specimens should not be processed in the routine specimen reception area.

Packaging and transport of biological specimens to sites outside the facility
For transport from the local pathology laboratory to NVRL specimens should be packaged in UN2814 certified packaging and transported according to UN620 guidance. Regulations regarding packaging and transport of Category A infectious substances are governed the ADR (European Agreement concerning the International Carriage of Dangerous Goods by Road) and International Air Transport Association (IATA) both of which use the United Nations Model Regulations system.
Personnel involved in packaging and sending samples are responsible for adhering to current regulations and interpreting applicable regulations for their facility. Appropriate certified training is recommended.
Each local laboratory should have appropriate packaging on site. Category A transfers should be individually requested through an approved courier. The courier must be licensed to carry dangerous goods and have appropriate training. The service should be available 24/7 and must involve tracked door-to-door delivery, which must be signed for on collection and receipt.
Specimens must be transported in triple packaging system according to the following instructions (Figure 1):

- primary containers must be leak-proof and a waterproof, leak-proof seal must be used;

- secondary packaging must also be leak-proof and contain sufficient absorbent material to absorb the entire contents of the primary container. If multiple primary containers are packaged together they must be individually wrapped to prevent contact;
- outer shipping packaging should be UN2814 certified (Packaging Instruction 620); specimen data forms, letters etc should be taped to the secondary container. Request forms are available at https://nvrl.ucd.ie/info.
- The box should contain a label "Infectious Substance". Write the name of the suspected microbe being transported in brackets.
- place the name, address and contact number of the destination laboratory (NVRL) on the outside of the box.
- place the name, address and contact number of the originator on the outside of the box.
- complete a transport document and provide a copy to the licensed courier.
- ship to the NVRL, University College Dublin, Dublin 4.

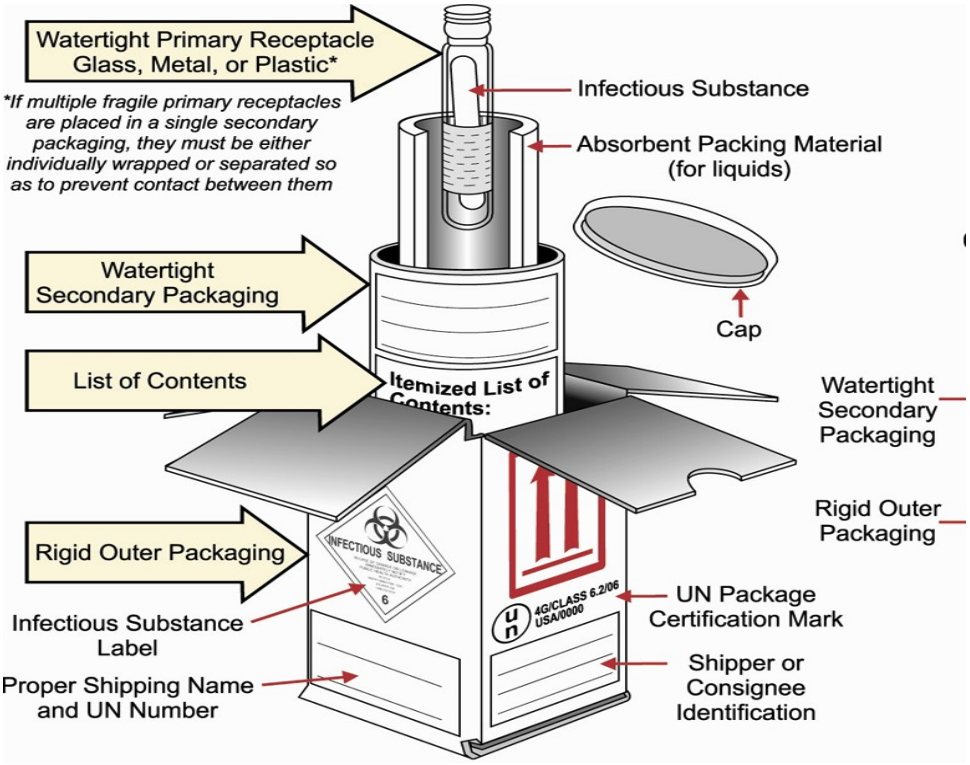


Figure 1: Example of UN2814 Packaging (Packaging and labelling as an infectious substance. Transporting Infectious Substances Safely, US DOT Document PHH50-0079-0706)

A licensed courier must be used for the transport of infectious or suspected infectious specimens.

Confirmation of molecular analysis and serological investigation, if required, is performed at the Rare and Imported Pathogens Laboratory, Porton Down, UK (RIPL). Transport of samples to the RIPL will be coordinated by the NVRL according to UN620 guidance.

Diagnostic testing for VHF viruses

- VHF testing is ONLY PERFORMED WITH PRIOR CONSULTATION WITH THE NVRL IN ACCORDANCE WITH THE HPSC ALGORITHMS and upon receipt of a completed VHF investigation request form.

- Preliminary VHF results will be reported within 24 hours. If the VHF screen is negative then the possibility of the patient having a VHF infection should be maintained until an alternative diagnosis is confirmed.

For all patient specimens with a risk of VHF, specific risk assessments must be developed alongside local codes of practice, which should be agreed between clinical and laboratory staff. This information can be used to ensure that the risks are effectively controlled and relevant facilities are in place and are managed properly. The risk assessment should include evaluation of the risks associated with each analytical technique and the application of appropriate control measures.
Any cause of fever must be considered in a febrile patient suspected of VHF infection; however, the major infectious microbial causes include malaria, shigellosis, typhus or typhoid fever. Most suspected VHF cases will subsequently be diagnosed as malaria. Therefore, upon presentation of a possible case of VHF in the ***At Risk*** category, malaria tests should be performed immediately. If the malaria test result is negative a VHF test should be considered.
Request forms for investigation of VHFs can be downloaded from the NVRL website (http://www.ucd.ie/nvrl/pdfs/BL3_Investigation_Request_Form_LF_UM_001d-3.0.pdf) and must be completed in full before testing can proceed. **TESTING IS PERFORMED ONLY WITH PRIOR CONSULTATION WITH THE NVRL.** Consultation is available 24/7 by calling +353 87 9806448. During normal business hours, the NVRL telephone number for all queries including **Clinical/Urgent Queries Telephone**: +353 1 716 4401.

Procedure for the Transport of Infectious Substance Category B

This covers most samples remitted to NVRL for testing

Category B
An infectious substance which does not meet the criteria for inclusion in Category A. Infectious substances in Category B shall be assigned to UN 3373.
The proper shipping name for Category B, UN3373 is "BIOLOGICAL SUBSTANCE" and must be packaged for carriage by road in accordance with ADR Packing Instruction P650

Specimens or samples suspected or known to contain Infectious substance Category B are packaged and transported in a three layer system (Figure 2)

- Specimen to be sent should be stored in a secure (preferably plastic) primary container.
- Wrap the container in tissue or cotton wool, which will act as absorbent material in event of any spillages.
- This should be placed in a biohazard bag
- Place the biohazard bag with the sample in a padded (jiffy bag) envelope
- Label the outer packaging with a hazard warning label, 'Diagnostic Specimen'
- Place the name, address and contact number of the destination laboratory (NVRL) on the outside of the box. **Please avoid the use of staples for the closure of packages as these present a safety hazard to NVRL laboratory staff.** Place the name, address and contact number of the originator on the outside of the envelope of transport container
- The specimen can be transported under the appropriate temperature conditions.
- UN3373 packaging can be used – as below, including the label 'Biological Substance, Category B'.

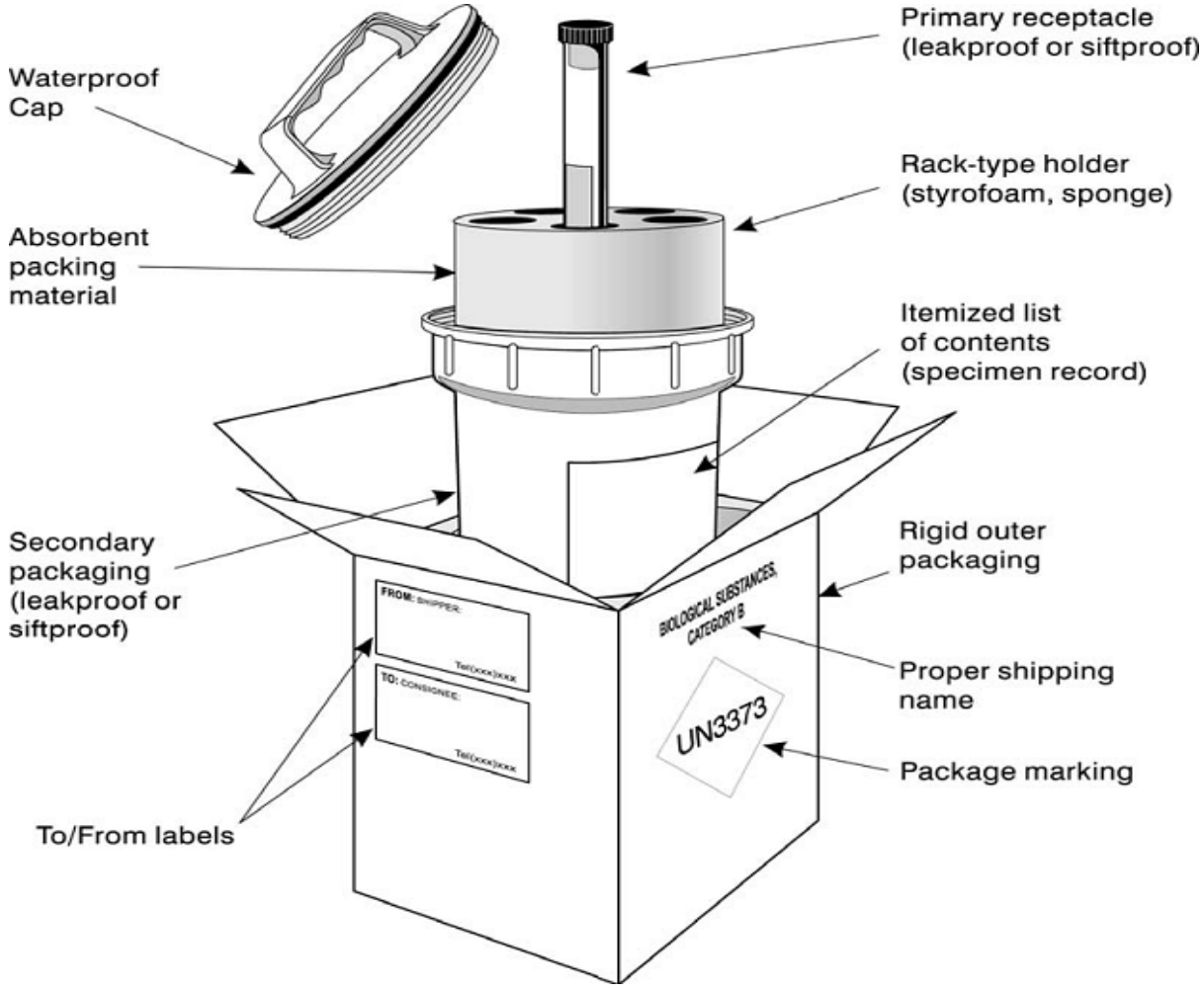


Figure 2: Example of UN3373 Packaging
(https://www.utmb.edu/bof/BC/Training/section9/5-TransBio.asp#5.4)

It is the responsibility of the consignor of infectious substances assigned to UN3373 to ensure that the packaging complies with all of the requirements of Packing Instruction P650. Consignors of UN 3373 may be nursing homes, doctors, dentists, the HSE (hospitals and primary care clinics), and personnel working in clinics, nursing homes, hospitals and surgeries must receive specific training in the correct classification of the infectious substances to be carried, and the application of P650.