



STANDARD OPERATING PROCEDURE

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1. Introduction

The Department of Virology (DOV) at Inkosi Albert Luthuli Central Hospital (IALCH) is an academic department in the National Health Laboratory Service (NHLS) in KwaZulu-Natal (KZN) and the University of KwaZulu-Natal (UKZN). We provide a diagnostic and clinical consultative service in KZN. This document provides information and guidance on the tests offered by DOV.

2. Purpose

The aim of this manual is to guide the diagnostic work-up of patients by providing criteria on proper specimen collection, handling, transportation and appropriate test selection which improves the quality of specimens received by DOV and facilitating patient management.

3. Scope

The scope of this document includes DOV contact details, specimen criteria, test repertoire, clinical indications for testing and Turn-around Times (TAT).

4. Responsibility

Users of DOV diagnostic and consultative service in KZN should familiarize themselves with the contents of this manual.

5. Abbreviations

BAL	Bronchoalveolar Lavage
CMV	Cytomegalovirus
CSF	Cerebrospinal Fluid
DBS	Dried Blood Spot
DOV	Department of Virology
EBV	Epstein-Barr Virus
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
HBcIgG	Hepatitis B Core Total Antibody
HBcIgM	Hepatitis B Core IgM
HBeAg	Hepatitis B e Antigen
HBsAg	Hepatitis B Surface Antigen
HBsAb	Hepatitis B Surface Antibody
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
HTLV	Human T-Cell Lymphotropic Virus
IALCH	Inkosi Albert Luthuli Central Hospital
KZN	KwaZulu-Natal
LIS	Laboratory Information System
NHLS	National Health Laboratory Service
N	Negative
P	Positive
PCR	Polymerase Chain Reaction

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PPT	Plasma Preparation Tube
SST	Serum Separation Tube
TAT	Turn-around Time
VTM	Viral Transport Medium
VZV	Varicella Zoster Virus

DOV Working Group: Dr K Francois, Dr L Gounder, Dr A Khan, Dr K Govender, Dr P Moodley, Dr NB Msomi

6. Physical Location

Level 5
Laboratory Building
Inkosi Albert Luthuli Central Hospital
800 Vusi Mzimela (Bellair) Road
Mayville

7. Contact Details

Reception Telephone: 031- 240 2599/2600
Clinical queries can be sent to dovkzn@gmail.com
After-Hours Telephone: 031-240 1000 – Virology Registrar on call

8. Operating Hours

DOV is open from Monday to Friday from 07h30 to 16h30. Please contact the on call DOV Consultant or Registrar for after-hours emergencies/consultation through the IALCH Switchboard on 031-240 1000. Calls will be forwarded to the relevant Registrar.

9. Quality Statement

DOV strives to provide quality results. We comply with ISO 15189 for Medical Laboratories through a Quality Management System and are accredited by SANAS.

10. Confidentiality

The NHLS has a policy on protection of personal information. All DOV staff sign a confidentiality agreement in which they agree to maintain patient confidentiality. Access to the Laboratory Information System (LIS) is denied to any person not employed by the NHLS in accordance with the NHLS IT Policies.

All results can be viewed electronically by the requesting clinician using the KZN DOH Intranet and /or the Internet (web access) which is user and password restricted to ensure confidentiality and protection of information.

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11. Specimen Submission

11.1 Specimen Collection

All specimens for Virology Tests must be sent to your local laboratory where specimen and patient details from request forms will be captured into the LIS. Your local laboratory will verify specimen and request form quality by following the criteria in section 11.2.

Test requests from IALCH must be done electronically using the Hospital Information System. These specimens must have a correctly placed barcode, placed into individual specimen packets and sent to us via the pneumatic tube system or by the IALCH porters. The specimens should reach us at least within 4 hours of collection. It is the responsibility of the person sending the specimen to ensure that the electronic request corresponds to the unique bar-coded specimen.

Patients should have verbal/written informed consent where appropriate before specimen collection. Standard safety precautions must be adhered to by all staff when collecting and handling any specimen. The appropriate personal protective equipment (PPE) must be used. All specimens must be collected using aseptic/sterile technique.

Any collection device and contaminated material used during specimen collection must be discarded appropriately into sharps containers and/or bio-hazard waste disposal boxes. The staff must adhere to the hospital protocols and their professional body Health and Safety regulations.

Each specimen must be placed in a specimen packet and the request form inserted into the separate pouch to prevent contamination if there is breakage and/or leakage of the specimen during transport.

All external (outside IALCH) specimens should be transported on ice and should reach the DOV at least within 72 hours. All specimens must be transported in accordance with the National Road Safety Act 6.2.

The local laboratory will send specimens to us where it is tested by us or sent away to other NHLS Laboratories for testing if we are unable to do the test.

All urgent or telephonically discussed specimens must be clearly indicated on the hard-copy request form/electronic request to facilitate the request.

Clinicians and referring laboratories will be informed by e-mail of any change regarding specimen collection requirements prior to the change.

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11.2 Request Form and Specimen Criteria

Tests will only be done on specimens accompanied by a correctly completed request form and adequate history. The test requested must be specific. Requests such as 'Viral Screen', 'Viral Hepatitis', 'Septic Screen' 'TORCH' etc will not be accepted. Please use the viral differential diagnoses provided in section 16 when requesting a test. All details on the request form and specimen must be legible. The minimum details required per patient request form are:

- A properly completed NHLS request form
- Patient surname and full name
- Legible clinical history
- Date and time of collection
- Gender
- Date of birth
- Exact name of Hospital/ward/clinic
- Patient hospital/clinic number
- Name, Signature and contact details of requesting Doctor or Nurse
- Exact name of test requested
- Specimen type

Specimens must be labelled properly and legibly with adequate information to link the correct specimen to the patient and request form. Specimens without proper identification will not be tested since it is a medico-legal hazard to test a specimen which does NOT link to an exactly identifiable patient.

Specimens from staff who sustained a needle-stick injury or splash must NOT be labeled with their name and surname. These specimens must have a confidential number according to the KZN DOH Policy.

11.3 Rejection Criteria

Specimens will not be tested for the following reasons:

- Any problem listed in Section 11.2 request form and specimen criteria
- Inappropriate clinical indication
- Incorrect specimen type, tube, container and transportation conditions
- Specimen leaking
- Expired tubes
- Old and/or haemolysed, Icteric, or Lipaemic specimens
- Incorrectly and/or incompletely labeled specimen
- Mismatched details between specimen and request form
- Illegible handwriting
- Insufficient specimen volume - at least 2 mL of specimen is required for most tests
- No test requested
- Problem with health care worker confidentiality (in context of occupational exposure)
- Test not offered

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12. DOV Test Repertoire and Requirements

Blood must be collected in a Serum Separation Tube (SST) – yellow or red-topped tube (tests requiring serum) or a Plasma Preparation Tube (PPT) - white-topped EDTA (tests requiring plasma) with separating gel. A separate tube must be sent for each test. Specimens should reach us on day of collection. If delays are expected, specimens should reach testing laboratory within 48-72 hours of collection and kept at 2-8°C during this time. Centrifugation of specimens by your local laboratory to separate plasma/serum from clotted blood prior to storage or transportation to us will prevent haemolysis and deterioration of the plasma/serum quality.

12.1 HIV Testing

Table 1: HIV Tests

Test	Clinical Indication, Specimen Type & Collection, Handling & Storage
HIV-1 Qualitative PCR	<p>HIV Early Infant Diagnosis – HIV exposed infants ≤ 24 months as per National Guidelines.</p> <p>DBS - preferred specimen type. Blood specimens prone to degradation while DBS is stable at room temperature.</p> <p>Blood from heel or finger/toe prick spotted onto filter card to fill entire spot, 3-5 spots required. DBS card must be left to dry on tissue paper or drying rack for at least 3 hours. Care must be taken not to allow cards to touch each other to avoid contamination.</p> <p>DBS cards should be stored & transported in a specimen packet with a desiccant sachet at room temperature. DBS has good long term stability if collected & stored appropriately. However, we still require timeous transport of DBS to prevent TAT delays.</p> <p>HIV PCR is not routinely done for adults. Discuss with us regarding HIV PCR for discrepant ELISA results.</p>
HIV-1 Viral Load	<p>Monitoring of patients on antiretroviral therapy as per National Guidelines.</p> <p>2-5ml blood must be collected in a PPT - Plasma</p>
HIV-1/2 Antibody/ Antigen ELISA	<p>Diagnosis in adults & children >18 months</p> <p>2-5ml blood must be collected in a SST (Serum)</p> <p>Specimen tube must be dedicated exclusively for HIV testing to prevent cross contamination.</p> <p>HIV-1/2 Rapid antibody test available at on site laboratories & after hours</p> <p>Screen, Confirmatory done if screen positive</p>
HIV Drug Resistance/ Genotyping	<p>Virological failure on Second Line ART in adults</p> <p>Virological failure on First Line and/or PI based in children</p> <p>2-5ml blood must be collected in a PPT (Plasma)</p>

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12.2 Viral Serology

Table 2: Viral Serology Tests

Test Name	Clinical Indication
Hepatitis A IgM	Acute Viral Hepatitis
HBsAg Rapid	Acute/Chronic Viral Hepatitis Available at on-site laboratories & after hours
HBsAg	Acute/Chronic Viral Hepatitis
Hepatitis C Antibody	Chronic Viral Hepatitis
HBsAb	Hepatitis B Immunity
HBcIgG	Hepatitis B markers are reflex tests which are added by DOV depending on sAg result.
HBcIgM	
HBeAg	
HSV IgM	Primary Infection
VZV IgM	Primary Infection
Rubella IgM	Pregnancy, Macular-Papular Rash, Congenital Rubella
Rubella IgG	Past infection & Immunity
CMV IgM	Serology used to diagnose current infection. IgM will be done first. An IgG will be done if IgM is positive to differentiate primary infections from reactivations.
CMV IgG	
EBV VCA IgM	Rubella IgG avidity will be added by DOV depending on serology & clinical indication.
EBV NA IgG	
Toxoplasma IgM	
Toxoplasma IgG	
HTLV-I/II IgG	Rare cause of Spastic Paralysis

12.3 Viral PCR

Specimen Requirements: Each specimen must be collected using aseptic/sterile technique. The lid of all specimen containers must properly tightened to prevent leakage and contamination. The specimen container must be labeled with the correct patient details which must match the request form properly. Specimens must be transported on ice, kept at 2–8°C and must reach the DOV at least within 48-72 hours of collection. Specimens which may be vulnerable to drying, can be placed in normal saline (e.g. respiratory/ulcer swabs, biopsies)

Table 3: Viral PCR Tests

Test	Specimen Type
HSV-1&2 PCR	Dependent on clinical features – ulcer/vesicle swab, CSF, eye fluid, EDTA whole blood
VZV PCR	Dependent on clinical features - ulcer/vesicle swab, CSF, eye fluid
CMV Viral Load	EDTA whole blood, BAL
CMV Qualitative PCR	Urine, eye fluid, biopsies
Parvovirus B19 PCR	EDTA whole blood
Polyomavirus JCV PCR	CSF
Respiratory Viruses Multiplex PCR	Respiratory Specimens
SARS-CoV-2 PCR	Respiratory Specimens

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Table 4: Specimen Collection Methods for PCR Molecular Testing

Specimen	Collection Method
Blood	EDTA (purple-topped) tube without gel PPT (purple-topped) tube with gel
Biopsies	Biopsy is inserted into a sterile universal container with normal saline
Bone Marrow	Bone marrow aspirate collected in an EDTA (purple-topped) tube.
Bronchoalveolar Lavage (BAL)	BAL is inserted into a sterile tube.
Cerebrospinal Fluid (CSF)	CSF is inserted into a sterile tube. Specimens must be sent in original container. Do not aliquot specimens. No VTM needed. CSF must be at least >0.5 ml. It must reach us within 24 hours of collection
Ulcer Swab	Remove a sterile Dacron swab (without gel) from container. Rub swab tip in lesion/ulcer in a circular motion and place tip into tube. Break off upper portion of swab and tighten container lid.
Nasal washings/ Naso-pharyngeal Aspirate	Use a sterile syringe to aspirate. Expel specimen into container and tighten container lid.
Stool	Place about 1 gram portion of stool into a sterile container. No VTM is needed. Tighten lid and secure properly.
Throat Swab	Remove a sterile Dacron swab (without gel) from container. Use a tongue depressor and good light. Rub swab tip against posterior pharyngeal wall of pharynx and place tip into tube. Break off upper portion of swab and tighten container lid.
Urine	Urine must be sent in a sterile universal container. Tighten lid and secure properly.
Vesicle Swab	Puncture vesicle, using a sterile needle, to disrupt roof of vesicle. Rub swab tip over lesion/ulcer in a circular motion and place tip into container. Break off upper portion of swab and tighten container lid.
Vesicle/Eye Fluid	Aspirate at least 0.2 ml vesicle/blister fluid using an insulin syringe. Release fluid into container and tighten lid. Do not leave needle and syringe in container. These should be discarded in an appropriate sharps container.

Other viral PCR tests are referred to SANAS accredited Virology Laboratories for testing. Highly specialized tests for referral e.g. HBV, HCV and EBV viral load testing will be arranged on a case-by-case basis after discussion with the Registrar or Consultant on call.

13. Turn-around Times

Turn-around-Time (TAT) from time of receipt of specimen in DOV to time of result is as follows:

- National Priority Programme tests - HIV-1 Qualitative PCR & HIV-1 VL: 4 days
- HIV ELISA: 7 days
- Other Viral PCR & Serology Tests: 14 days
- HIV DR: 30 days

Any additional test request on specimens must be requested within at least 3 days of specimen collection (Table 5). All additional requests must be discussed telephonically with the DOV Registrar on call. An urgent request e.g. viral PCR on CSF will be done urgently depending on clinical indication as decided by the attending Doctor and Registrar on call.

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Table 5: Storage Times of Specific Specimens

Test	Duration	Comment
Viral Serology	7 days	Discarded
HIV-1 Qualitative PCR	DBS stored for 1 month	DBS then discarded. Whole blood discarded immediately after testing.
HIV-1 Viral Load	Not stored	Discarded after testing.
Viral PCR	7 days (depends on specimen volume)	CSF may be stored longer.

If a repeat test on a primary specimen is required due to a possible analytical error or further tests are required, then the requesting clinician will be informed on the final report.

14. Referral of Specimens to other Laboratories for testing (Send-away Tests)

A test which is not done by the DOV will be requested from another Laboratory in the NHLS. All tests on specimens from these patients must be discussed with the DOV to enable clinical advice regarding the need for the specific test. The TAT will depend on the other Laboratory. Generally results will be made available electronically at least within 25 working days, unless urgent. The results for specimens from patients with suspected Viral Haemorrhagic Fever will usually be available within 24-48 hours depending on the clinical urgency.

15. Notifiable Medical Conditions

The following conditions due to viruses are Notifiable Medical Conditions (NMC):

1. Acute Flaccid Paralysis (AFP)
2. SARS-CoV-2
3. Congenital Rubella Syndrome – Clinical Features and Rubella IgM positive in first 3 months
4. Measles – Macular-Papular rash, cough, fever, conjunctivitis and Measles IgM and/or urine PCR positive
5. Rubella - Macular-Papular rash and Rubella IgM positive
6. Viral Hepatitis
7. Rabies
8. VHF – any viral cause

16. Tables of Viral Differential Diagnosis

Table 6: Viral Hepatitis

All Viral Hepatitis are notifiable medical conditions.

Viral Causes	Tests offered
Hepatitis B	See Table 7 for HBV Serological markers HBV load used to monitor patients with chronic HBV
Hepatitis C	Screen with HCV ELISA. HCV viral load used for confirmation of positive serology & monitoring patients on treatment. HCV genotyping prior to treatment initiation.
Hepatitis A	HAV IgM - Used for investigation of outbreaks of acute hepatitis

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Table 7: Serological Markers of HBV Infection and Interpretation

HBsAg	HBeAg	HBcIgM	HBcIgG	HBsAb	Interpretation
P	N	N	N	N	Early Infection
P	P/N	P	P	N	Acute Infection
N	N	P	P/N	N	Diagnostic Window
P	P	P/N	P	N	High Infectivity
P	N	N	P	N	Carrier/Chronic *
N	N	N	P	P	Immune, Past Infection
N	N	N	N	P	Immune due to vaccination or recent HBIG

*Persistent detection of HBsAg for >6 months indicates chronic hepatitis. N=Negative, P=Positive.

Table 8: CNS Viral Infections

Viral causes of CNS diseases are usually uncommon. Please discuss with DOV Registrar on call prior to ordering. There is usually no specific drug treatment except for Herpesviruses (HSV, CMV, VZV). PCR is done only on CSF except for AFP which requires stool.

Diagnosis	Possible Viral Causes to consider
Aseptic Meningitis	Enteroviruses, Mumps
Encephalitis	HSV - Suggest starting IV acyclovir empirically Consider CMV & VZV in immunocompromised patients Rabies, Measles, Viral Zoonoses (Table 15)
Myelitis	Enteroviruses, VZV, HTLV Associated Myelopathy, HIV Myelopathy
Progressive Multifocal Leukoencephalopathy (PML)	JC Virus
Acute Flaccid Paralysis (AFP)	AFP is an NMC. Any cause of flaccidity in one or more limbs in children <15 years eg transverse myelitis due to any cause. Last Polio case in South Africa was in 1989. AFP Surveillance is NOT looking for specific case due to Polio. Two stool specimens collected 24 hours apart and within 14 days of AFP onset must be sent for AFP surveillance. Specimens must be accompanied by AFP Case Investigation Form.

Table 9: Viral Infections of the Eye

Diagnosis	Possible Viral Causes	Comment
Conjunctivitis Keratoconjunctivitis Scleritis	Adenovirus Enteroviruses HSV, VZV, Measles	Conjunctival swab for HSV, VZV PCR Investigation of outbreaks - conjunctival swab for Adenovirus, Enterovirus PCR.
Retinitis	CMV, VZV, HSV, HIV	Vitreous fluid for HSV, VZV, CMV PCR

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Table 10: Gastroenteritis

Diagnosis	Viral Causes	Comment
Childhood Diarrhoea Epidemic diarrhoea	Rotavirus Adenovirus Calicivirus Astrovirus	It is not useful to do tests for gastroenteritis. Testing is only necessary as part of outbreak investigation, after discussion with DOV/NICD. There is no antiviral treatment available

Table 11: Viral Infections of Skin and Mucosa

Rash	Viral causes	Tests offered
Macular-papular	Measles Rubella Parvovirus	Measles is an NMC. Blood and urine specimens for surveillance must be sent to NICD with Measles Case Investigation Form.
Petechial Rash	Viral Zoonoses(Table 15)	Rubella: Blood for Serology. Pregnant women < 20 weeks gestation with signs & symptoms of rubella or exposure to Rubella/"rash-illness" should be investigated. Please contact DOV.
Vesicular &/or ulcerative	VZV HSV	Vesicle & ulcer swabs for PCR. (Table 4).

Table 12: Respiratory Infections

Suspected Diagnosis	Viral Causes	Comments
Influenza-like illness- fever, cough, myalgia, fatigue, pharyngitis	SARS CoV-2 Influenza Parainfluenza Adenovirus RSV Rhinovirus	Testing done based on Clinical Guidelines only Testing is recommended for severe acute respiratory infections. Respiratory specimens include:
Pneumonia (especially in infants, elderly & immunocompromised)	SARS CoV-2 Influenza, RSV, Adenovirus, Parainfluenza , CMV, VZV, HSV, Measles	<ul style="list-style-type: none"> • Endotracheal Aspirates (ETA) • Bronchoalveolar Lavages (BAL) • Nasopharyngeal Aspirates (NPA) • Throat Swabs • Lung Biopsy
Bronchiolitis & Laryngotracheo-bronchitis (LTB, Croup)	RSV Parainfluenza Adenovirus Influenza	Measles is an NMC. Blood and urine specimens for surveillance must be sent to NICD with Measles Case Investigation Form.

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Table 13: Viral infections in Pregnancy and Neonate

Diagnosis	Viral Causes	Tests Offered
Pregnant women < 20 weeks gestation with signs & symptoms of Rubella or exposure to Rubella/"rash-illness"	Rubella Parvovirus	Rubella – SST blood for IgM & IgG. If positive, please contact DOV for further advice to facilitate clinical decisions. Parvovirus – PPT/EDTA blood for PCR
Congenital Abnormalities (e.g. microcephaly, cataracts, deafness)	Rubella Toxoplasma VZV CMV	Investigation of neonate should be directed by peri-natal history & clinical findings. Positive IgM in first 2-3 months of life suggests an intrauterine infection. Not useful at all after 3 months. Congenital Rubella Syndrome is a NMC
Foetal hydrops or persistent, severe neonatal anaemia	Parvovirus	PPT/EDTA blood for PCR
Pregnant woman with severe pneumonia	Influenza VZV SARS-CoV-2	Respiratory specimen for multiplex respiratory PCR. VZV PCR can be done on vesicle fluid & respiratory specimens if chickenpox suspected.
Neonatal Sepsis	HSV Enteroviruses CMV Rubella VZV Toxoplasma	Viruses are not common causes of neonatal sepsis. Blood for Serology and/or PCR testing depending on clinical features Urine CMV PCR is only useful in first 3-4 weeks of life. Thereafter, urine CMV PCR positive may represent shedding of postnatal acquired CMV which is not clinically significant. These requests will be cancelled if there is lack of a proper clinical indication.

Notes:

- There is no antiviral treatment available for most viral infections in pregnancy.
- Viruses do NOT cause Bad Obstetric History or Recurrent Abortions.
- These tests should not be used for screening ie NOT part of 'Septic Screen'
- These tests are indicated for ill patients who require a diagnosis and further management.

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Table 14: Viral Infections in Immuno-compromised Patients from Haematology, Oncology, Transplant Units

Diagnosis	Viral Cause	Comment
GVHD/Post-Transplant Patient	CMV EBV	Pre-transplant serology usually done in graft recipients & donors to establish sero-status & risk for reactivation/infection. Viral load can be done post-transplant as a pre-emptive treatment strategy.
Pneumonitis	CMV VZV Adenovirus Respiratory Viruses	Rising CMV Viral loads post-transplant pre-empts disease
Hepatitis	Hepatitis A,B,C CMV	Hepatitis serology & molecular testing (Table 8 & 9). Rising CMV Viral loads post-transplant pre-empts disease
Haemorrhagic Cystitis	BK Virus Adenovirus	Urine PCR testing is indicated. Please contact DOV
Nephropathy post renal transplant	BK Virus CMV	BK viraemia is a better predictor for development of BK nephropathy than detection in urine. BK viral load is recommended.
Post-Transplant Lymphoproliferative Disorder	EBV	Rising EBV viral loads post-transplant is suggestive

Table 15: Suspected Viral Haemorrhagic Fever (VHF) and Viral Zoonosis

Syndrome	Viral causes to consider	Comments
Fever, Arthritis, Rash with or without Encephalitis	Dengue Rift Valley Fever West Nile virus Zika virus	Must have a history of travel to an endemic area, contact with known cases, or with livestock etc & compatible clinical findings. Specimens must be sent directly from patient bedside to NICD.
Viral Haemorrhagic Fever	Crimean Congo Haemorrhagic Fever (CCHF), Ebola virus, Rift Valley Fever, Dengue, Lassa Virus, Yellow Fever	Testing done based on Clinical Guidelines only.
Encephalitis	Rabies Virus	All suspected cases must be discussed with DOV Registrar on call to facilitate patient investigation. Contact NICD Hotline: 082 883 9920 Viral Haemorrhagic Fever and Human Rabies are NMCs.

Note: Clinician and Peripheral Laboratory must contact DOV for advice on Clinical and Laboratory Infection, Prevention & Control

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17. Laboratory Results

Any urgent result such as for VHF will be communicated **as soon as possible** to the Clinician by DOV.

All results can also be searched for and viewed electronically using the KZN DOH Intranet and /or the Internet (web access). This access is **user and password** restricted to ensure confidentiality and protection of information. The access can be arranged by application to your Peripheral Laboratory Manager.

Requesting clinicians may contact DOV **R**egistrar on call for assistance or advice on patient investigation and result interpretation.

18. Compliments and Complaints

Please use **our** contact details listed in **S**ection 7, or alternatively, e-mail our Quality Assurance Supervisor at neshni.ramjugath@nhls.ac.za.

19. References

NHLS Handbook - GPQ0064 - Working Group, Quality, **P**atience **D**abula

Acknowledgement of Reading Form

Document number: _____ **Version Number:** _____

Title: _____

My signature confirms that I have read and understood the content of this document and relevant kit insert (where applicable).

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