



**INVESTIGATION OF ARBOVIRAL NEUROLOGIC & HAEMORRHAGIC DISEASE,
 ABORTIONS & CONGENITAL DEFORMITIES, AND VIRAL RESPIRATORY DISEASE IN FARM AND WILD ANIMALS**

ZRU REFERENCE NUMBER		DATE	
Name of owner of animal		Cell nr	
Owner email address		Tel nr	
Location: disease occurred		GPS	
Name of referring veterinarian		Cell nr	
Practice name and address		Tel nr	
Vet email address		Fax nr	
Name/ID of animal		Sender Ref	
Species	Breed	Sex	Age
Cloven hoofed animals*	Yes/no	*Must be submitted through Transboundary animal diseases (TAD)/OVR under red cross permit to ZARV	
Requested test:			
DATE SPECIMENS WERE TAKEN:		FIRST SUBMISSION	OR FOLLOW UP
Specimen type	Blood samples: <input type="checkbox"/> EDTA <input type="checkbox"/> Clotted blood	<input type="checkbox"/> Animal Alive	<input type="checkbox"/> Animal Dead
Postmortem	<input type="checkbox"/> Neurological signs: <input type="checkbox"/> Brain	<input type="checkbox"/> Spinal chord	<input type="checkbox"/> CSF
Samples on ice	<input type="checkbox"/> Respiratory signs: <input type="checkbox"/> Lung	<input type="checkbox"/> Liver	<input type="checkbox"/> Spleen
Date of death	<input type="checkbox"/> Died	<input type="checkbox"/> Euthanized	<input type="checkbox"/> Abortion/Fetus/Stillborn
Other Details:			
CLINICAL SIGNS	DATE ONSET OF CLINICAL SIGNS:		
<input type="checkbox"/> Fever _____°C	<input type="checkbox"/> Anorexia	<input type="checkbox"/> Anaemia	<input type="checkbox"/> Icterus
Neurological Signs	<input type="checkbox"/> Ataxia	<input type="checkbox"/> Paresis	<input type="checkbox"/> Hindleg Paralysis
<input type="checkbox"/> Head Tilt	<input type="checkbox"/> Nystagmus	<input type="checkbox"/> Tongue Paralysis	<input type="checkbox"/> Paddling
Respiratory Signs	<input type="checkbox"/> Congested mucous membranes	<input type="checkbox"/> Cyanotic mucous membranes	<input type="checkbox"/> Nasal discharge
Respiratory Rate	<input type="checkbox"/> Cough	<input type="checkbox"/> Dyspnoea	<input type="checkbox"/> Pulmonary Edema
Other Clinical Signs	<input type="checkbox"/> Abortion	<input type="checkbox"/> Foetal Deformity	<input type="checkbox"/> Arthrogyposis
		<input type="checkbox"/> Hepatitis	<input type="checkbox"/> Rectal Prolapse
		<input type="checkbox"/> Foreleg Paralysis	<input type="checkbox"/> Recumbent
		<input type="checkbox"/> Seizures	<input type="checkbox"/> Blindness
		<input type="checkbox"/> Haemorrhagic manifestations	
(continue on back of form)			
Treatment for Current Disease			
Were specimens submitted to another laboratory for diagnosis?		<input type="checkbox"/> OVI <input type="checkbox"/> ERC <input type="checkbox"/> Other(specify):	
<input type="checkbox"/> African Horse Sickness	<input type="checkbox"/> Equine encephalosis	<input type="checkbox"/> Equine Herpes 1 and 4	<input type="checkbox"/> Rabies
<input type="checkbox"/> Brucella	<input type="checkbox"/> Bacterial culture	<input type="checkbox"/> Other (specify):	
Recent vaccinations	<input type="checkbox"/> AHS1 <input type="checkbox"/> AHS2	<input type="checkbox"/> Equine Flu	<input type="checkbox"/> Tetanus
Date last vaccination			<input type="checkbox"/> Rabies
			<input type="checkbox"/> West Nile Virus
Other (specify):			
<p>These investigations are performed as part of a surveillance and research programme and offered free of charge for collaborators. Specimens will be screened for relevance and quality. Only specimens accompanied by a fully completed ZARV submission form, that reach us on ice within 3 days of collection or frozen will be tested by PCR. Specimens must be taken within 10 days of onset of illness for PCR and virus isolation; Specimens taken > 10 days will be used for serology. Virus isolation cannot be performed if frozen at -20 °C. Serology and virus isolation results will be reported retrospectively if applicable or available only.</p> <p>Submit specimens to: Prof Marietjie Venter, Centre for Viral Zoonoses, Room 2-72, Pathology Building, 5 Bophelo Rd, Cnr Steve Biko and Dr. Savage, University of Pretoria Prinshof Campus, Pretoria 0001. Contact: Prof Marietjie Venter +27(0)123192638; +27(0)832930884, marietjie.venter@up.ac.za; or zarvrp@gmail.com, +27(0)12 319 2329 or Rebecca Jeal 0844282288 (see separate information on taking of specimens and packaging).</p> <p>NB: Please send specimens PACKAGED ON ICE by courier or via other laboratory services, not by post. Please use appropriate containers –no syringes or needles (see information sheet). All cases submitted to this program need to be notified to the state vet before it will be tested. Specimens from Cloven hoofed animals need to be submitted to TAD/OVR for RNA extraction under red cross permit and nucleic acids send to ZARV for testing (Attention Dr Livio Heath for Prof M. Venter) and the state vet notified as per signature below.</p> <p>We will share the results with the state vet if a controlled or notifiable animal diseases in terms of the Animal disease act, 1984 (Act No 35 of 1984) is detected as well as any other positive results. The test results remain the intellectual property of the CVZ and will be used in scientific publications. The data cannot be used for publication by others without our consent. The identity of the animal and owner will not be revealed in scientific publications. The CVZ reserves the right to NOT test samples that arrive in poor condition and not sampled within viraemic phase. The CVZ requests also that it may follow-up telephonically or via email on cases where the CVZ viral PCRs proved negative in order to record if another diagnosis was made and may request a follow up specimen for research purposes.</p>			

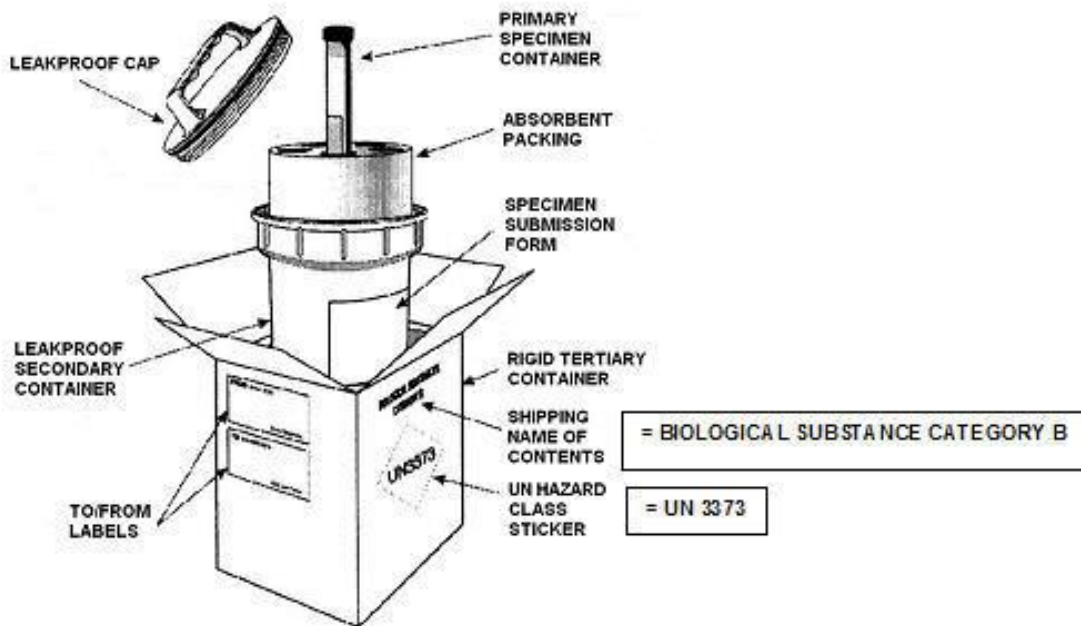
I _____ (submitter) confirm that I have personally notified the state veterinarian, Dr _____ email _____/cellphone _____ district _____ of this sample submission as the symptoms may be indicative of a suspected incidence of a controlled or notifiable animal disease in terms of the Animal disease act, 1984 (Act No 35 of 1984)".

Signature of acknowledgement of submitter: _____ Date: _____

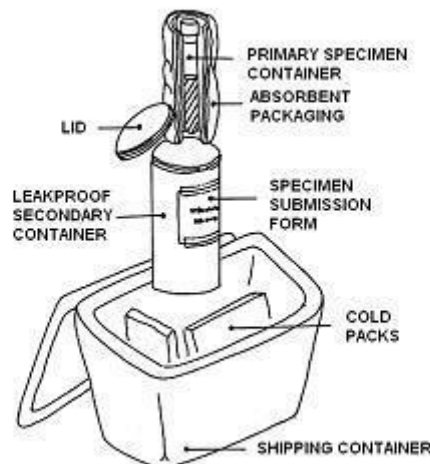
PACKAGING FOR TRANSPORT OF BIOLOGICAL SUBSTANCES,

NB: SEND SPECIMENS BY COURIER OR VIA OTHER LABORATORY SERVICES, NOT BY POST, WHICH IS ILLEGAL AND RESULTS IN DELAYS THAT RENDER SPECIMENS UNSUITABLE FOR TESTING.

Commercially available biosafety packaging e.g. Aulax (RSA), Saf T Pak (Canada) that conforms with IATA Regulations



Example of Improved Biosafety packaging





**Zoonotic Arbo and Respiratory virus Program
Centre for Viral Zoonoses (CVZ)
Department Medical Virology**

INFORMATION SHEET: INVESTIGATION OF NEUROLOGICAL DISEASE IN FARM AND WILD ANIMALS

Background. The Zoonotic arbo and respiratory virus program (ZARV) in the Centre for Viral Zoonoses (CVZ) investigate whether West Nile and other **arthropod-borne viruses (arboviruses:** viruses transmitted by blood-sucking arthropods such as mosquitoes, midges, sandflies and ticks), including Sindbis, Middelburg, Wesselsbron and Shuni viruses, account for cases of undiagnosed fatal and/or neurological disease in farm animals such as horses and cattle, as well as in wild animals such as rhinoceroses, buffaloes, warthogs, giraffes and crocodiles. Monitoring neurological disease, unexplained fatalities as well as abortion or fever outbreaks act as an early warning system of annual outbreaks or emerging and re-emergence arboviruses or new zoonotic viruses as part of a One Health approach. By submitting specimens for investigation, you become a collaborator of the program and agree that we can use this in our research into zoonotic arboviruses. The ZARV may also investigate vectors or human cases around detected animal cases.

Research tests consist principally of molecular procedures (RT-PCR) to detect viral genetic material (nucleic acid) in blood, brain, or spinal cord samples. A DAFF compliant biosafety level 3 (BSL3) laboratory in the CVZ makes it possible to grow live virus from specimens in safety and identify and characterise emerging viruses. During acute illness the presence of virus is detected by RT-PCR, culture and virus discovery methods and later in the disease the diagnosis is established by demonstrating an IgM immune response.

The ZARV program specifically investigates zoonoses (diseases that humans can acquire from animals) and does not provide a general diagnostic service for veterinary pathogens, particularly controlled or notifiable diseases in terms of the Animal disease act, 1984 (Act No 35 of 1984). These diseases should be excluded by submitting specimens to the appropriate DAFF approved and SANAS accredited veterinary laboratories, with whom we collaborate.

All cases submitted to ZARV need to be accompanied by the DAFF approved submission form signed by the submitting veterinarian that the statevet had been notified that a specimen was submitted to ZARV with symptoms that may be indicative of a suspected incidence of a controlled or notifiable animal disease in terms of the Animal disease act, 1984 (Act No 35 of 1984)". Please confirm this was done by signing the submission form prior to submitting the sample. All specimens of cloven-hoofed animals need to be submitted directly to the Transboundary Animal disease program (TADP) at OVR under a red-cross permit for inactivation or RNA extraction with the completed ZARV submission form before nucleic acids will be sent to the ZARV. Send to TAD for attention Dr Liveo Heath for Prof Venter. A veterinary import permit have to be obtained from DAFF for specimens submitted from countries outside of South Africa on a cases to case basis and the samples imported in compliance with the conditions of the import permit.

NB Include case details as requested on the accompanying submission form: species, age, sex, dates of onset, sampling and death, clinical signs, geographic location, plus contact details of persons from whom further information can be obtained, and to whom results can be reported. Indicate whether duplicate specimens have been submitted to a veterinary laboratory for the diagnosis of controlled and notifiable animal diseases **in terms of the Animal disease act, 1984 (Act No 35 of 1984)** such as African horse sickness and rabies.

Additional specimens: Since neurologic disease may only be the extreme manifestation of infection, arrangements can be made to test blood samples from additional animals with milder signs on affected properties as part of the survey but the primary indication for testing is as stated above (neurological, fatalities, abortion not just single mild fever cases). The viruses are endemic, and detection of an IgG antibody titre alone is indicative of past infection but is not evidence of current or recent infection. This necessitates a follow up specimen for IgM testing for specimens outside of the viremic period where virus RNA can be detected or to investigate a rise in antibody levels. For cases that test positive or negative by PCR we may also request a follow up blood for WNV IgM confirmation.

Costs. Investigations are performed free of charge and funded by research grants, which have been sourced by the principle investigator. The investigations may however be costly and for this reason, only appropriate specimens that fit the case definition and that were taken during the correct phase of disease will be accepted. Insurance cases or healthy animals due for export should be discussed with the head of the program, Prof Venter and the appropriate forms used.

Specimens. Wear appropriate protective clothing (gloves, gown/apron, mask, goggles) when performing autopsies.

Live animals with neurologic disease: EDTA plus clotted blood can be taken and sent on ice packs. If possible, spin down the clotted tubes by centrifugation before shipping.

Fatal cases: Submit 2cm³ blocks of relevant brain and cord tissue with viral transport medium in separate labelled containers with ice (see attached diagrams). Alternatively, submit caudal quadrant (includes cerebrum, midbrain, cerebellum and brainstem) plus spinal cord (especially lumbar) and blood (e.g. cardiac puncture), sent with ice packs. Visceral organs may also be submitted for further investigations.

Unidentified virus isolates or specimens from veterinary pathology laboratories can also be submitted if accompanied by our submission form. **Safety and quality control regulations require rejection of specimens that are degenerated/decomposed, or arrive in broken, leaking or otherwise unsuitable primary containers (syringes, plastic bags).**

Send specimens to: Prof Marietjie Venter, Centre for Viral Zoonoses, Room 2-72, Pathology Building, 5 Bophelo Rd, Cnr Steve Biko and Dr Savage, University of Pretoria Prinshof Campus, Pretoria 0001

Contact: Prof Venter: +27(0)123192282; marietjie.venter@up.ac.za; ZARP or zarvrp@gmail.com, or +27 (0)12 319 2329 or Rebecca Jeal 0844282288 to arrange for specimens to be received (see attached information on packaging). **NB: send specimens by courier or via other laboratory services appropriate for specimen handling on ice and correctly packaged within 48 hours not by mail as legally required for infectious specimens. Avoid unnecessary delays that reduce specimen quality and practices that have a biosafety risk. Specimens to be sent in clearly marked tubes, indicating material type and preservative - no needles or syringes allowed.** Questions can be directed to Prof Marietjie Venter at +27 (0)832930884, marietjie.venter@up.ac.za. For autopsies or histopathology on farm animals contact Dr June Williams, Section Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, +27 (0)832348886, June.Williams@up.ac.za, and for wild animals Dr Johan Steyl at the same address, +27 (0)823984823, Johan.Steyl@up.ac.za.