Rubén Villalba (LCV–Spain OIE Ref. Laboratory for AHS)
Carrie Batten (Pirbright-UK OIE Ref. Laboratory for AHS)
Alan Guthrie

Webinar #2: AHS diagnostic tests and possible support from the OIE Reference Laboratories

INDEX

1. AHS diagnostic tests
2. Support from the OIE Ref. Labs
3. Regional Support
4. Diagnosis: final recommendations

1. AHS diagnostic tests

African horse sickness diagnosis

Clinical and post-mortem diagnosis

Clinical sign and histological lesions are **not pathognomonic**.

Even oedema of the supraorbital fossae could be present in other infectious disease such as Equine Encephalosis.

Picture from experimental infection.
LCV. 9 dpi

Therefore, **laboratory diagnosis is essential to differential diagnosis** to other infectious diseases, such as, Equine Encephalosis, Equine Infectious Anaemia, Morbillivirus Pneumonia, Equine Viral Arteritis, Babesiosis or Purpura Haemorrhagica.

African horse sickness virus

RELEVANT PROTEINS IN DIAGNOSIS

VIRAL PROTEIN	GENOME SEGMENT
VP1	1
VP2	2
VP3	3
VP4	4
VP5	6
VP6	9
VP7	7
NS1	5
NS2	8
NS3-NS3A	10
NS4	9

VP2: -the **most variable protein** among serotypes.
-an **haemoagglutinin** directly involved in the processes of adhesion of the viral particle to the host cell.
-the principal antigen that determines the formation of **serotype-specific seroneutralizing antibodies**.

VP7: -**highly conserved** protein across the serotypes
-a **serogroup-specific** antigen

Reassortment can occur when two viruses (of the same species) co-infect a single host cell, generating **novel virus phenotypes**, with potentially dramatic biological consequences, including an altered ability for immune escape, changes in host or vector range, changes in transmissibility and altered virulence or pathogenicity

African horse sickness diagnosis (sampling)

The outcome of a diagnostic test is strongly influenced by the **sample quality**, the **storage conditions** and the **way in which the sample is collected**.

DIRECT DIAGNOSIS (Agent detection)

- In live animals, **whole blood** should be collected with anticoagulant (EDTA for PCR)
- In dead animals the following should be collected: **spleen, lungs, lymph nodes and blood**

INDIRECT DIAGNOSIS (Antibody detection)

Serum sample

African horse sickness diagnosis (sampling)

All samples collected must be maintained at a **temperature of +4°C** and analysed in the laboratory as soon as possible. They may be kept at 4°C up to a week.

For agent detection:

Blood must always be conserved and transported refrigerated, **never frozen**. Blood sample freezing causes the virus release associated to the erythrocyte membrane, resulting in its neutralisation by the antibodies present in the plasma.

Organs: if they cannot be sent to the laboratory immediately, there must be stored at -80°C. Never frozen at -20ºC.

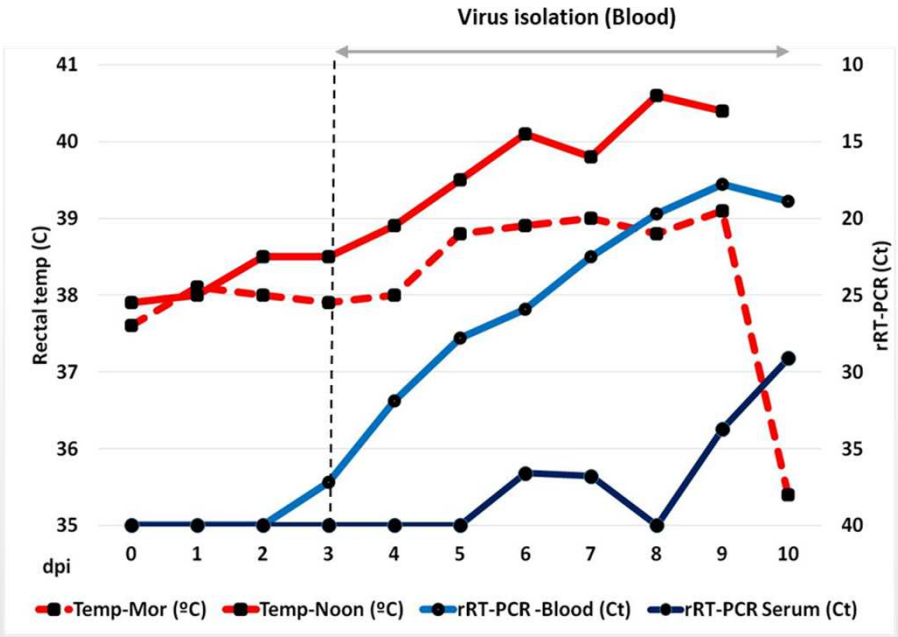
For serology:

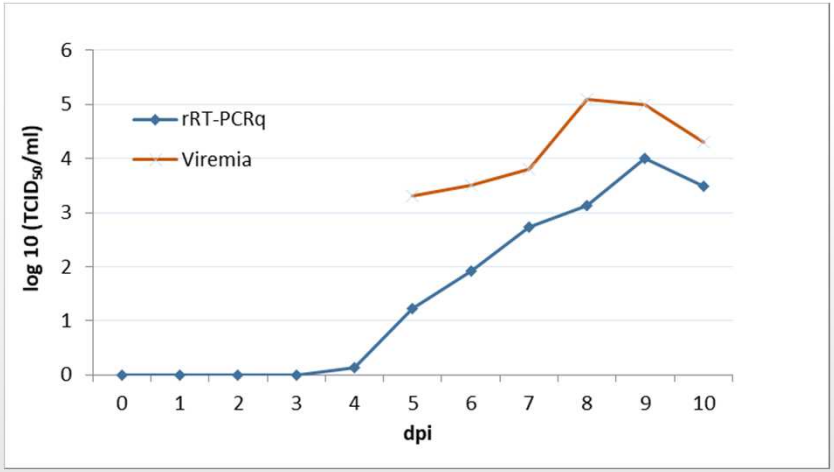
Sera must be kept at +4°C. If they cannot be sent to the laboratory immediately, the sera must be stored at -20°C.

African horse sickness diagnosis (diagnosis)

RELEVANT PARAMETHERS DURING AN EXPERIMENTAL INFECTION

LCV 2018. Unpublished





Euthanized

ANTIBODY DETECTION AT 10 dpi
ELISA + SNT+

Period of viraemia: short in horses (4–8 days)
Thompson et al. 2012 donkeys may remain viraemic for up to four weeks
zebra can extend up to around 40 days

African horse sickness diagnosis (diagnosis)

DIRECT DIAGNOSIS (Agent detection)
Methods according to OIE Manual

3) AHSV strain further characterization
- Sequencing

2) AHSV serotype identification
- RT-PCR serotype-specific (seg-2)
- VNT (virusneutralization test)

1) Serogroup (AHSV) detection
- RT-PCR (serogroup-specific)
- Virus isolation in cell culture

Other agent detection techniques, such as Sandwich ELISA, have been usually replaced by RT-PCR

African horse sickness diagnosis

RT-PCR (serogroup-specific)

DIRECT DIAGNOSIS (Agent detection)
Methods according to OIE Manual

1) Serogroup (AHSV) detection
- RT-PCR (serogroup-specific)
- Virus isolation in cell culture

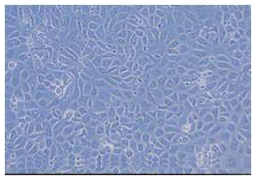
Although several gel-based and real-time RT-PCR methods have been described targeting different conserved viral segment (3, 5, 7 and 8), **real-time RT-PCR methods of Agüero *et al.* (2008) and Guthrie *et al.* (2013)** are validated for certification of individual animals prior to movement and for confirmation of clinical cases (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals).

African horse sickness diagnosis

Virus isolation in cell culture

DIRECT DIAGNOSIS (Agent detection)
Methods according to OIE Manual

1) Serogroup (AHSV) detection
- RT-PCR (serogroup-specific)
- Virus isolation in cell culture



Cell cultures (**BHK21, VERO, MS**) are infected with the material under examination and observed for at least 7 days for a cytopathogenic effect (CPE).

- Although blood samples could be used directly as the inoculum, **it is recommended to wash and lyse erythrocytes to remove unwanted antibodies** which could neutralise free virus, **and to promote releasing of virus** associated with the red blood cell membranes.
- In tissue samples, a **10% tissue suspension** is prepared in PBS or cell culture medium, containing antibiotics.

CPE is an unspecific event and a **confirmation** by using other method such as virus neutralization test or RT-PCR is required.

Intravenous inoculation of embryonated chicken eggs and intracerebral inoculation of newborn mice are **alternative methods** for virus isolation.

African horse sickness diagnosis

DIRECT DIAGNOSIS (Agent detection)
Methods according to OIE Manual

2) AHSV serotype identification
- RT-PCR serotype-specific (seg-2)
- VNT (virusneutralization test)

The rapid identification of the serotype is important to allow **proper planning of vaccination**.

RT-PCR: type-specific gel-based RT-PCR (**Maan et al., 2011; Sailleau et al., 2000**), and real-time RT-PCR (**Koekemoer, 2008; Bachanek-Bankowska K., et al. 2014; Weyer et al. 2015; Van Schalkwyk, A. et al. 2019; EURL unpublished**) targeting AHSV Seg-2 for identification and differentiation of AHSV genotypes, provides a rapid typing method for AHSV in tissue samples and blood.

The **genetic variation** that may appear over time in the AHSV genome, in particular in the VP2 coding region, would make difficult the detection of all genetic variants within each serotype by this type of technique. For that reason is **highly recommended to assay new circulating AHSV strains** to update these methods.

VNT: Once the **virus has been isolated**, it is tested against the different antisera being neutralised by the serotype specific antiserum. This technique **takes 5 or more days** before results are obtained.

African horse sickness diagnosis

DIRECT DIAGNOSIS (Agent detection)
Methods according to OIE Manual

3) AHSV strain further characterization
- Sequencing

Sequencing

The phylogenetic studies of AHSV have been performed using **whole or partial sequences** of the 10 segments of AHSV genome.

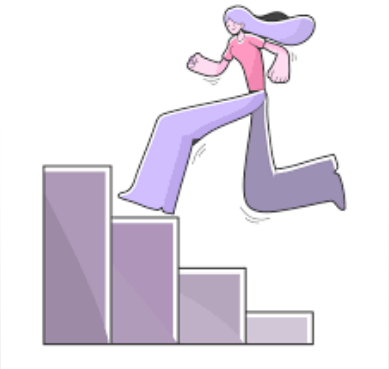
Segments 2 (VP2) and 10 (NS3) are the most variable AHSV genome segments and have been used in most of studies.

However to monitor **reassortment events and the presence of vaccine strains** it is recommended that complete genome sequences of representative sample should be obtained.

NGS technology allows to for rapid **whole genome** sequencing.

African horse sickness diagnosis

INDIRECT DIAGNOSIS
(Antibody detection)
Methods according to OIE Manual



2) AHSV antibody serotype identification

- SNT (seroneutralization test)

1) Serogroup (AHSV antibody detection)

- Indirect ELISA
- Blocking ELISA (commercially available)

Other serological techniques for serogroup detection, such as immunoblotting or Complement fixation , have been replaced by ELISA.

Detection of immune response methods are the recommended method for these purposes:

- ❑Population freedom from infection in non vaccinated populations
- ❑Prevalence of infection – surveillance in non vaccinated populations
- ❑Immune status in individual animals or populations post-vaccination

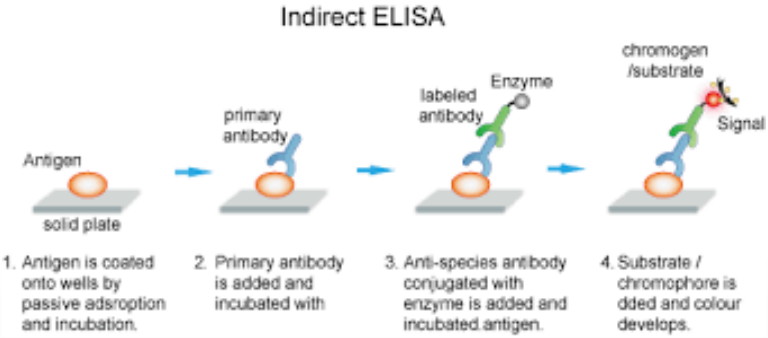
African horse sickness diagnosis

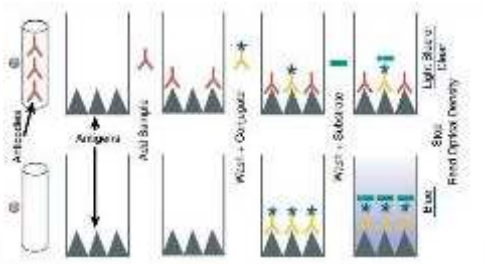
ELISA

INDIRECT DIAGNOSIS (Antibody detection)
Methods according to OIE Manual

1) Serogroup (AHSV antibody detection)

- Indirect ELISA
- Blocking ELISA (commercially available)





Blocking ELISA concept

Indirect and competitive blocking ELISAs using either soluble AHSV antigen or a **recombinant protein VP7**

It is very useful for large-scale investigations, although it does **not allow differentiation of antibodies from the different AHS serotypes**.

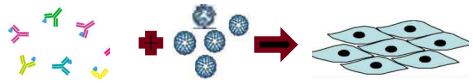
It does **not allow differentiation of infected and vaccinated** (VLA vaccines commercially available) **animals.**

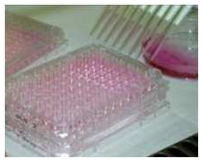
The reproducibility of the **blocking ELISA (Ingezim AHS Compac Plus)** was assessed in an OIE ring trial (Durán-Ferrer et al., 2018)

INGENASA
INgezim AHSV Compac PLUS
R.14.AHS.K3

African horse sickness diagnosis

SNT (Seroneutralization test)





INDIRECT DIAGNOSIS (Antibody detection)
Methods according to OIE Manual

2) AHSV antibody serotype identification

- SNT (seroneutralization test)

The need to work with live virus and the time required to obtain the results, 5 days, **do not allow SNT to be used as a routine test**.

SNT detects **neutralizing antibodies (serotype-specific against VP2),** while most of ELISAs detect **antibodies against VP7** (it is recombinant antigen coated in plates).

There must be considered that in vaccinated animals (VLA) there is a marked **variation in SNT titres between serotypes** (in case of polyvalent vaccine) **and between animals (even absent)**. The duration of neutralizing antibodies is often **transient** and it is currently **uncertain as to how precisely SNT titres correlate to protective immunity**.

African horse sickness diagnosis

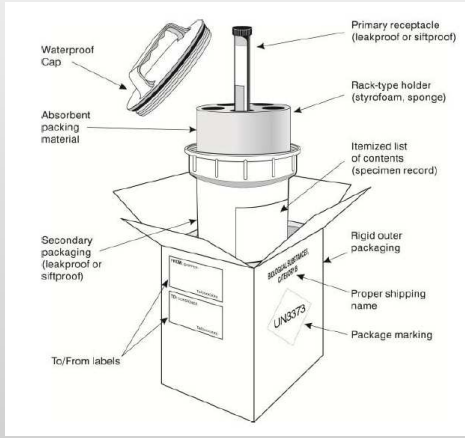
Biosafety in laboratory and transport of samples / cultured AHS virus

According to the OIE Manual Chapter 1.1.4 a **Laboratory risk assessment** should be used to identify biosafety and biosecurity measures needed.

Due to the severe economic consequences of disease presence, **CDC Manual** recommend to handle AHSV in a **Level-3 facility in countries where it is a foreign pathogen**.

Moreover, there must be considered the risk of the samples which come from countries where **other diseases** are endemic.

TRANSPORT: According to the IATA and ADR rules for transport of infectious material, **AHS virus both clinical samples and cultures**, are considered as **CATEGORY B UN3373**



2. Support from the OIE Reference Laboratories

OIE Reference Laboratories for AHS (4)

Dra. Montserrat Agüero Garcia
Laboratorio Central de Sanidad Animal
LCV-Algete, Ctra. Algete Km 8
28110 Algete (Madrid)
ESPAÑA
Tel: +34 913 47 83 12 Fax: +34913 29 05 98
Email: maguerog@mapa.es

Dr Simon Carpenter
The Pirbright Institute
Ash Road, Pirbright
Woking, Surrey, GU24 0NF
UNITED KINGDOM
Tel: +44-1483 23 24 41 Fax: +44-1483 23 24 48
Email: simon.carpenter@pirbright.ac.uk

Dr José Manuel Sánchez-Vizcaíno
Centro de Vigilancia Sanitaria Veterinaria
Facultad de Veterinaria
HCV Planta sótano
Universidad Complutense de Madrid (UCM)
Avda Puerta de Hierro s/n
28040 Madrid
ESPAÑA
Tel: +34-91 394.40.82 Fax: +34-91 394.39.08
Email: jmvizcaino@visavet.ucm.es

Dr Baratang Alison Lubisi
Onderstepoort Veterinary Institute
Agricultural Research Council
Private Bag X05
Onderstepoort 0110
SOUTH AFRICA
Tel: +27-12 529 91 17
Email: lubisia@arc.agric.za

African horse sickness diagnosis

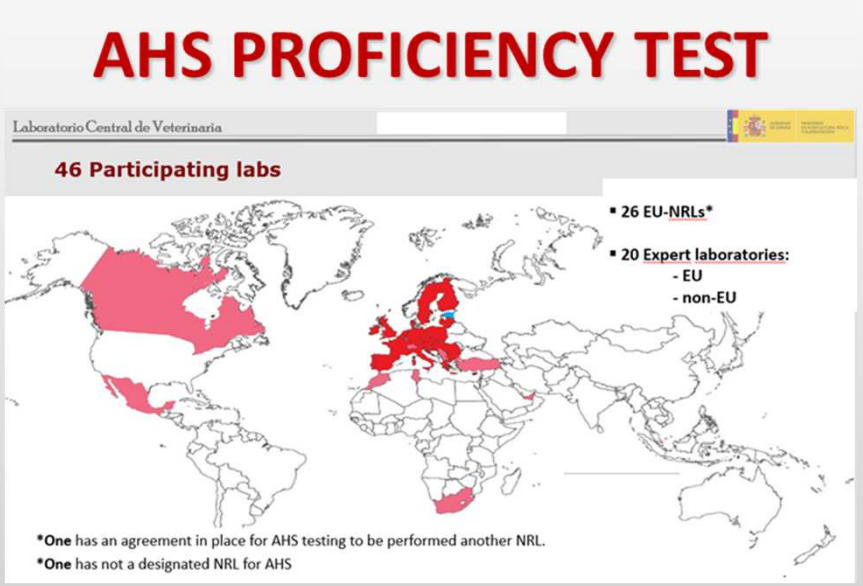
OIE Reference Laboratory: capacity and support

GOBIERNO DE ESPAÑA
MINISTERIO DE AGRICULTURA, PESCA Y ALIMENTACIÓN
Laboratorio Central de Veterinaria

As **EU Reference Laboratory for African horse sickness and Bluetongue**, LCV organize an annual AHS Proficiency Test, for serogroup diagnostic tool (ELISA and RT-PCR).

Singapore is the only country from Asia included in our laboratory network.

We would be happy to **invite other Asian laboratories to participate**, in order to help establish the main serogroup diagnostic tools according to the ISO 17025 accreditation.



African horse sickness diagnosis

OIE Reference Laboratory: capacity and support

GOBIERNO DE ESPAÑA · MINISTERIO DE AGRICULTURA, PESCA Y ALIMENTACIÓN

Laboratorio Central de Veterinaria

Technical advice and diagnostic service support, including serological and agent detection and characterization:

-Serology (ISO 17025): ELISA and Seroneutralization Test

-Agent detection (ISO 17025): serogroup/serotype qRT-PCR and virus isolation

-Agent further characterization: sequencing

In the LCV we handle AHSV samples in a **level 3 facility** with capacity to analyse a **high amount** of samples.

Disease OIE expert:
Montserrat Agüero

List of AHS reference material, Laboratory methods (SOPs) and Guidelines to the best implementation and quality control according to the ISO 17025 are available in the LCV website:

https://www.mapa.gob.es/en/ganaderia/temas/laboratorios/referencia-union-europea-oie/

The Pirbright Institute

The Pirbright Institute is a world leading centre of excellence in research and surveillance of virus diseases of farm animals and viruses that spread from animals to humans.

- Contributing to global food security and health, improving quality of life for animals and people
- Annual income >£29M (1.2 billion NT$)
- Over 180 scientists working on a range of viruses in specialist areas
- CL2 and CL3 laboratory space
- OIE CL4 containment facility (BSL3)
- High-containment (BSL3) animal facilities

Plowright building, Pirbright

www.pirbright.ac.uk

Reference laboratories at Pirbright

Vesicular disease reference laboratories

Non-vesicular disease reference laboratories

OIE reference laboratory for AHSV

- **Disease expert:** Dr Simon Carpenter – Head of entomology
- **Diagnostics:** Dr Carrie Batten (Head) and Dr John Flannery (technical manager)
- Technical advice and diagnostic service/support: ISO/IEC 17025 accreditation
- Real-time RT-PCR, Serotyping, virus isolation and characterisation (full NGS pipeline established)
- Training providers, with opportunities at Pirbright and/or in country
- Experience in delivering successful OIE twinning projects

carrie.batten@pirbright.ac.uk
alan.guthrie@up.ac.za
rvillalba@mapa.es

Laboratorio Central de Veterinaria

WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

12, rue de Prony, 75017 Paris, France
www.oie.int
media@oie.int - oie@oie.int