

Peste des petits ruminants ***(Laboratory Diagnostics – an overview)***

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IAEA

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

FAO/IAEA Joint Center



- On 1 October 1964, the **FAO** and **IAEA** created the Joint FAO/IAEA Center as a **strategic partnership** in order to mobilize the talents and resources of both organizations and hence to broaden cooperation between their Member States in the peaceful application of nuclear science and technology in a safe and effective manner to provide their communities with more, better and safer food and agricultural produce while sustaining natural resources.

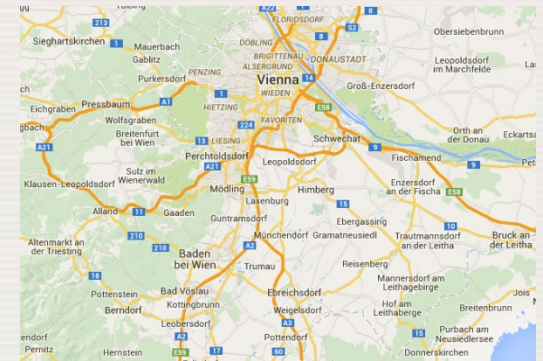
Five Laboratories

Soil and Water Management and Crop Nutrition
Plant Breeding and Genetics
Animal Production and Health
Insect Pest Control
Food and Environmental Protection

FAO/IAEA Joint Division

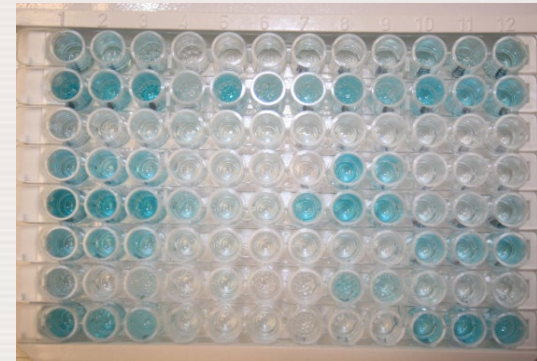


- The Animal Production and Health Laboratory (APHL) assists Member States to improve livestock productivity through the efficient use of locally available feed resources, adequate management practices and breeding programmes for indigenous and upgraded animals, and **diagnostic tools and prophylactic measures for the control and prevention of animal and zoonotic diseases.**



Diagnosis of PPRV

- Virological/Molecular/Serological
- Lab-based/Field-based



Samples

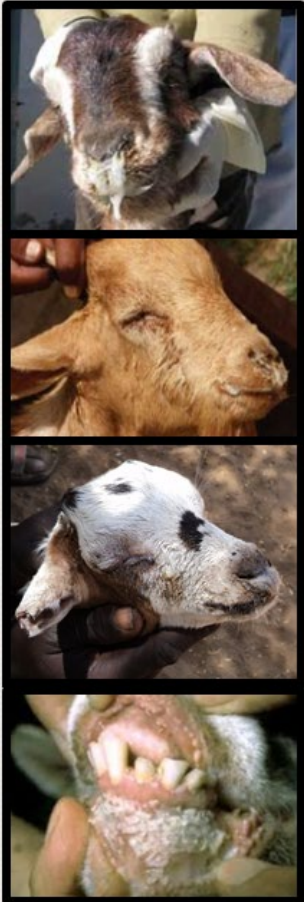
•Samples

For virus isolation, antigen capture and molecular diagnostics

- (1) Swabs of the conjunctival/nasal discharges and from the nasal and buccal mucosae
- (2) upon necropsy aseptically collect lymph nodes (especially the mesenteric and bronchial nodes), spleen and lung
- (3) Feces (Bataille et al Sci Rep. 2019 Mar 18;9(1):4742)
- (4) Bone marrow from carcasses

For serologic needs: clotted blood

For Histology: 10% Formalin



Samples - Transport

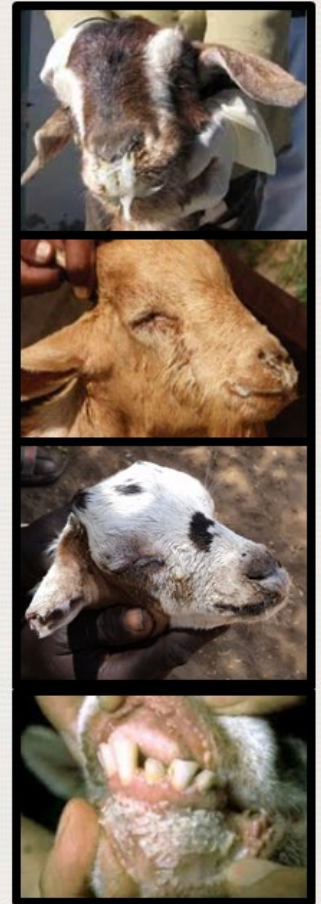
- Swabs (Ocular and Nasal) and organs can be transported “dry” or in Viral Transport Medium on ice/coolers to the lab.
- Filter paper is a simple and cost-effective transport medium for serological diagnosis of Peste des petits ruminants. (Torsson et al 2019 Small Ruminant Research 170, 154-159).
- Sera samples on ice/coolers (liquid nitrogen ?) (Fernandez Aguilar et al Viruses. 2020 Mar 7;12(3):293)



Virological

Virological

- Virus Isolation
- Antigen Capture
- Agar gel immunodiffusion (AGID)



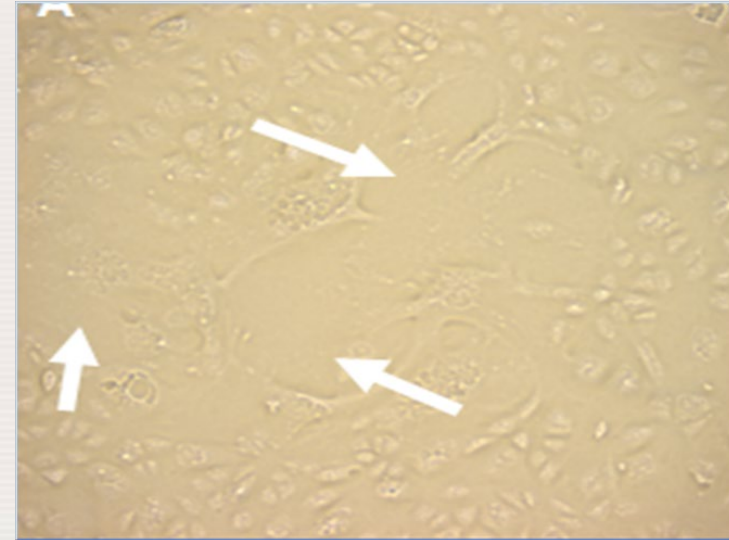
Virological (Cell culture)

- Important for definitive confirmation of presence of virus, infection studies and in-depth genetic characterization.
 - Primary Kidney Cells (Ovine or Bovine)
 - Vero
 - Madin-Darby bovine kidney (MDBK)
 - MDBK-Nectin-4
 - BHK-21
 - Vero-dog SLAM
 - CV1-SLAM



Virological (Cell culture)

- Signalling lymphocyte activation molecule (SLAM)
- Monkey CV1 cells expressing goat SLAM
- CHS-20 (available on request)



Journal of Virological Methods 173 (2011) 306–313

Contents lists available at ScienceDirect

 **Journal of Virological Methods**

journal homepage: www.elsevier.com/locate/jviomet



Monkey CV1 cell line expressing the sheep–goat SLAM protein: A highly sensitive cell line for the isolation of peste des petits ruminants virus from pathological specimens

Caroline Mélanie Adombi^a, Mamadou Lelenta^a, Charles Euloge Lamien^a, David Shamaki^b, Yao Mathurin Koffi^c, Abdallah Traoré^d, Roland Silber^e, Emmanuel Couacy-Hymann^c, Sanne Charles Bodjo^a, Joseph A. Djaman^f, Antony George Luckins^a, Adama Diallo^{a,*}

Virological (Cell culture)

Table 2

Results of the PPRV isolation trial on cell culture from the pathological specimens that were tested PPRV positive by classical and quantitative RT-PCR. CHS-20, CV1 and Vero were used in this trial.

Tissue	Sample ID	PPRV RT-PCR result on tissue sample	Virus isolation ^a			Detection of PPRV in cell culture supernatant (RT-PCR)		
			CHS-20	CV1 ^b	Vero ^b	CHS-20	CV1	Vero
Lung	NIG/08-03	+	3 days	N	N	+	-	-
	NIG/08-17	+	8 days	N	N	+	-	-
	NIG/08-27	+	2 days	N	N	+	-	-
	NIG/08-43	+	2 days	N	N	+	+	+
	CIV/09-OVP	+	N	N	N	-	-	-
	CIV/09-01P	+	1 day	N	N	+	+	.. ^c
	CIV/09-02P	+	2 days	N	N	+	-	-
Lymph node	NIG/08-04	+ ^d	N	N	N	-	-	-
	NIG/08-10	+ ^d	7 days	N	N	+	-	-
	NIG/08-30	+	3 days	N	N	+	-	-
	CIV/09-OVG	+	N	N	N	-	-	-
	CIV/09-01G	+	2 days	N	N	+	.. ^c	-
Liver	NIG/08-20	+	N	N	N	-	-	-
	NIG/08-28	+	5 days	N	N	+	-	-
	NIG/08-44	+	8 days	N	N	+	-	-
	CIV/09-OVF	+	N	N	N	-	-	-
	CIV/09-01F	+	N	N	N	-	-	-
	CIV/09-02F	+	4 days	N	N	+	-	-
Spleen	NIG/08-11	+ ^d	10 days	N	N	+	-	-
	NIG/08-18	+	10 days	N	N	+	-	-
	NIG/08-22	+	8 days	N	N	+	-	-
	NIG/08-29	+	7 days	N	N	+	-	-
	CIV/09-OVR	+	N	N	N	-	-	-
	CIV/09-01R	+	N	N	N	-	-	-
	CIV/09-02R	+	N	N	N	-	-	-
Kidney	CIV/09-02N	+	N	N	N	-	-	-

N = no CPE. Samples in bold were used in the RT-PCR shown in Fig. 3.

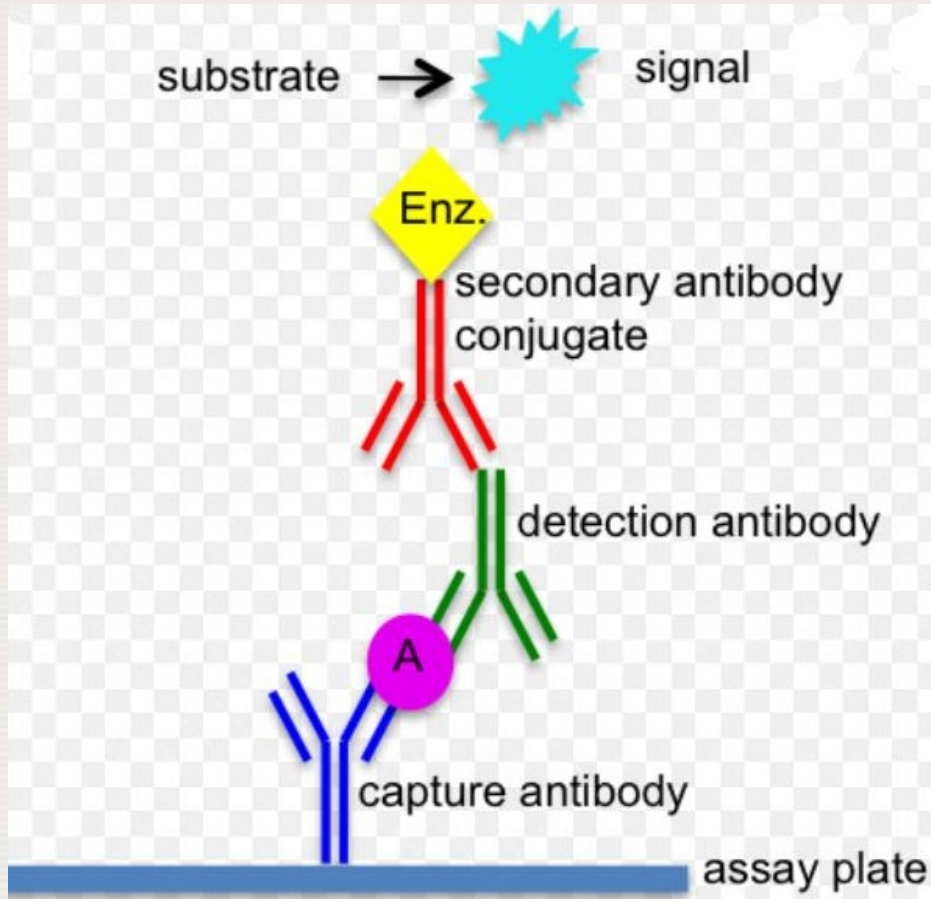
^a Day post infection (dpi) on which the CPE was detected in the cell monolayer.

^b Up to 4 blind passages were performed.

^c Positive for the RNAs that were extracted from the cells at the time of the first blind passage.

^d Positive in real time PCR only.

Virological (Antigen capture ELISA)



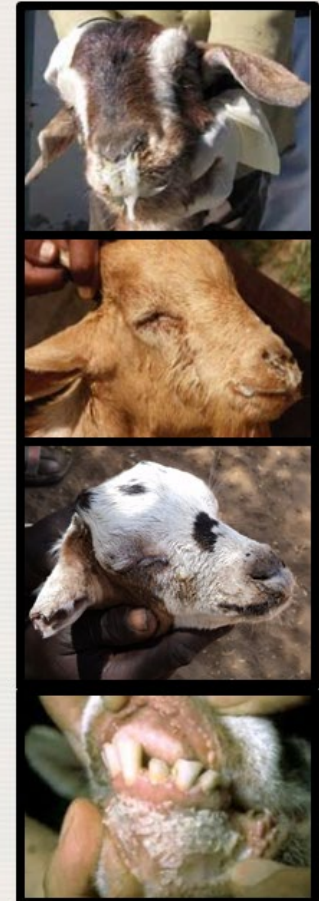
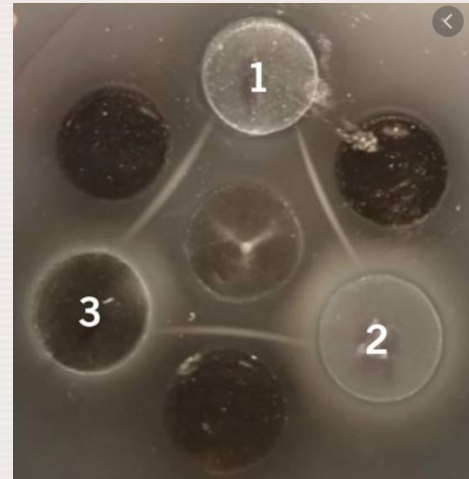
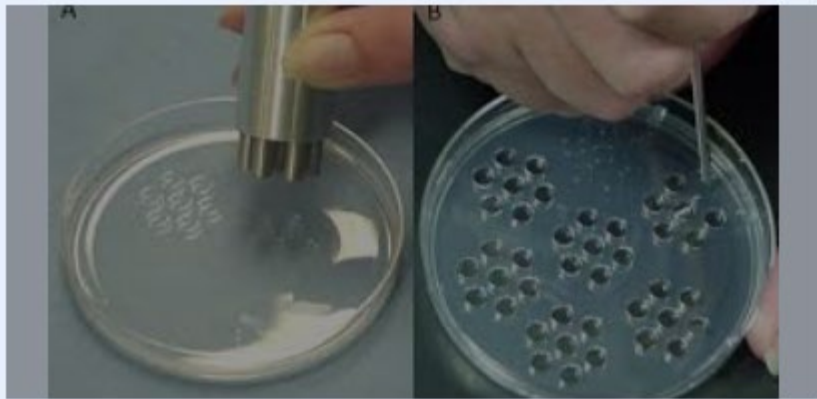
- aka Sandwich ELISA
- 96-well plate format
- Equivalent performance to RT-PCR



Virological

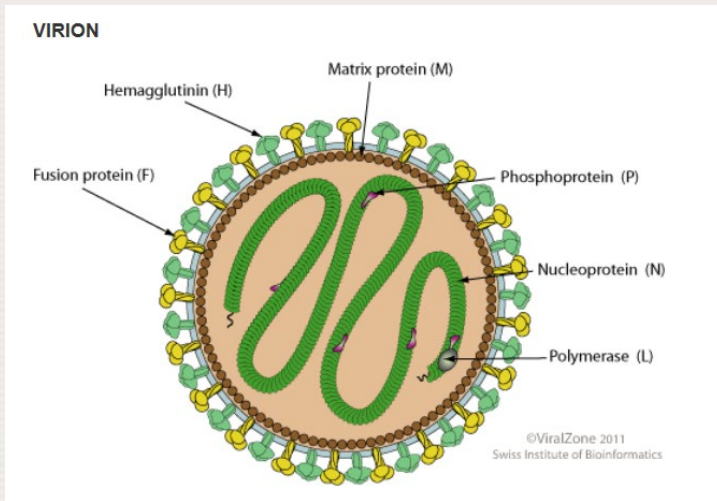
Agar gel immunodiffusion

- simple and inexpensive test that can be performed in any laboratory and even in the field
- standard PPR viral antigen is prepared from mesenteric or bronchial lymph nodes, spleen or lung material
- results are obtained in one day, but the test is not sensitive enough to detect mild forms of PPR due to the low quantity of viral antigen that is excreted

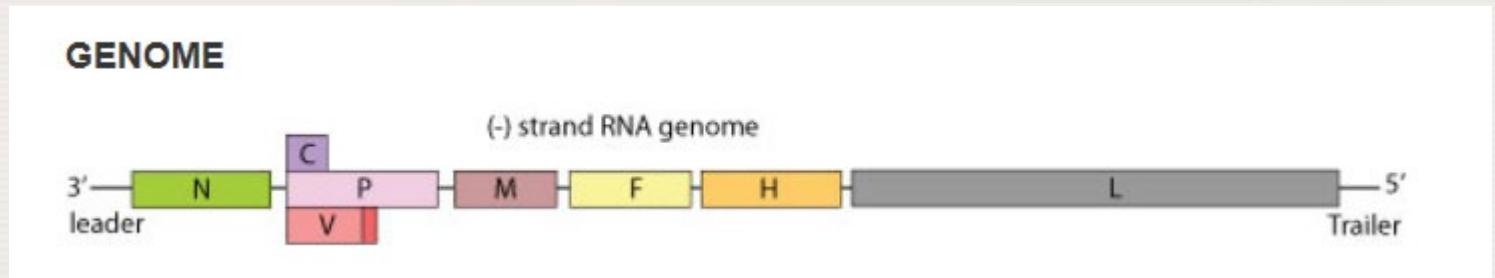


Molecular diagnostics

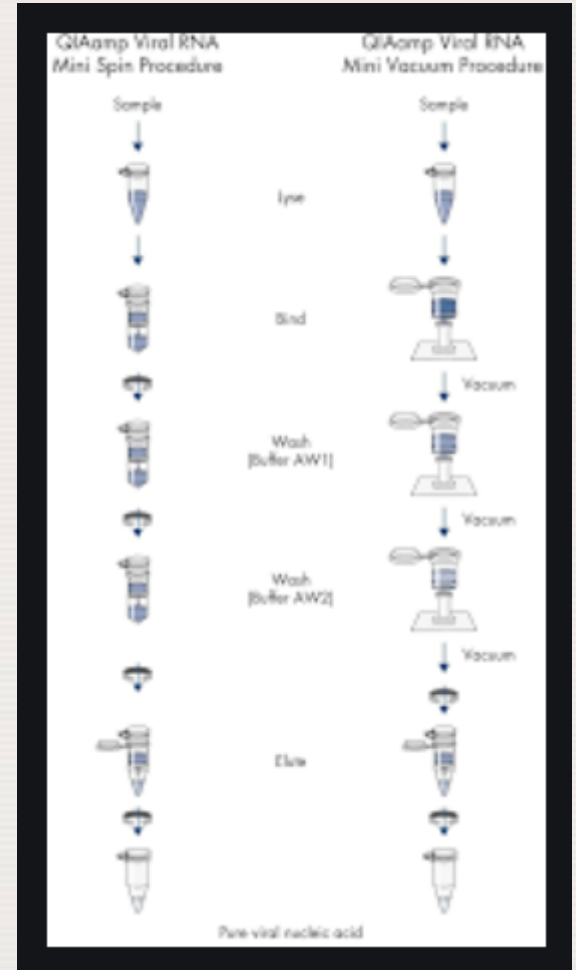
PPRV – genomic structure



- Morbillivirus (Measle, Rinderpest, CDV)
- Enveloped, non-segmented (-) RNA
- Approx. 16 Kb
- Encodes 8 proteins
- Single serotype

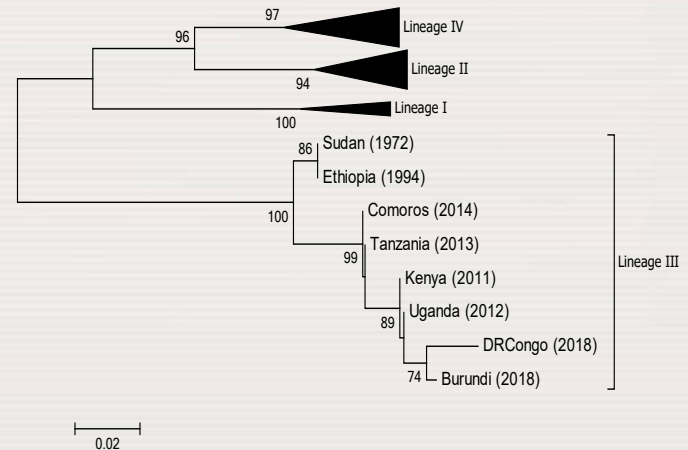
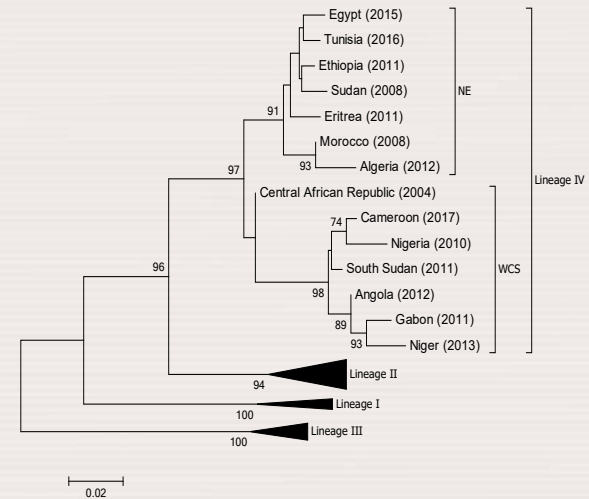
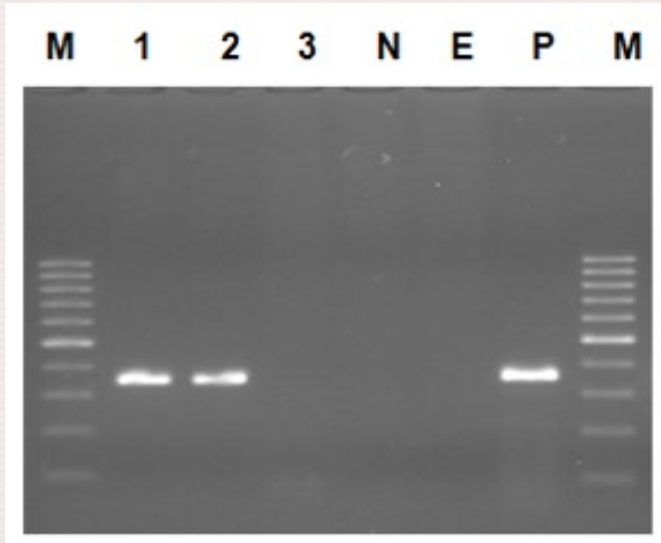


RNA purification



Conventional RT-PCR

- Primers
 - NP3/NP4 (Nucleoprotein) – 351 bp amplicon
 - F1/F2 (Fusion protein) – 343 bp



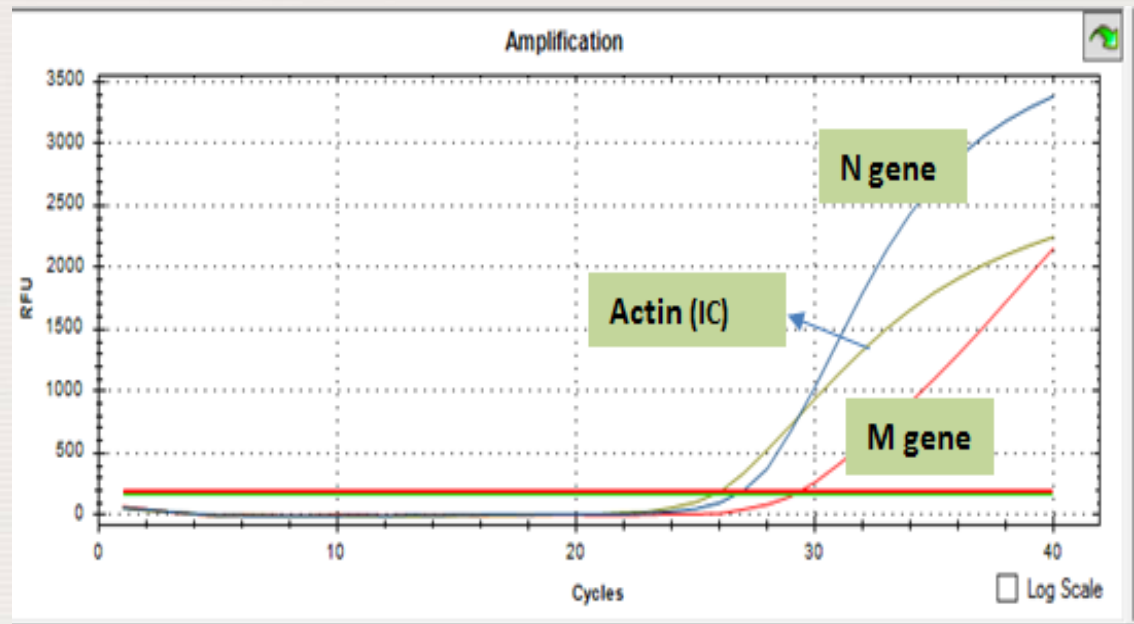
Quantitative Reverse Transcription – PCR (qPCR)

Many choices available

- Lucas J, et al A versatile dual-use RT-PCR control for use in assays for the detection of peste des petits ruminants virus. J Virol Methods. 2020 Mar;277:113799.
- Flannery J et al F-gene RT-qPCR assay for the detection of all four lineages of peste des petits ruminants virus. J Virol Methods. 2019 Dec;274:113735
- Batten CA, et A real time RT-PCR assay for the specific detection of Peste des petits ruminants virus. J Virol Methods. 2011 Feb;171(2):401-4.
- Abera T et al A SYBR Green I based real time RT-PCR assay for specific detection and quantitation of Peste des petits ruminants virus. BMC Vet Res. 2014 Jan 14;10:22.
- Bao J, et al. Development of one-step real-time RT-PCR assay for detection and quantitation of peste des petits ruminants virus. J Virol Methods. 2008 Mar;148(1-2):232-6.
-

RT-qPCR

Internally controlled assay targeting two genes two PPRV genes:



Multiplex assays

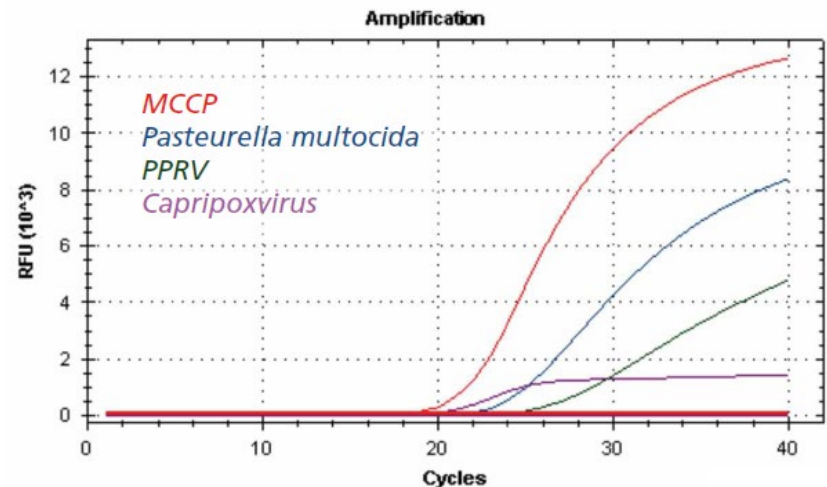
- Diseases
- Contagious caprine pleuropneumonia (MCCP)
- Pasteurellosis
- PPRV
- Capripox

Share a number of common clinical signs and, importantly, can occur as co-infections

RESEARCH ARTICLE

One-Step Multiplex RT-qPCR Assay for the Detection of *Peste des petits ruminants virus*, *Capripoxvirus*, *Pasteurella multocida* and *Mycoplasma capricolum subspecies (ssp.) capripneumoniae*

Tirumala Bharani Kumar Settypalli^{1*}, Charles Euloge Lamien¹, Joachim Spergser², Mamadou Lelenta¹, Abel Wade³, Esayas Gelaye^{1,4}, Angelika Loitsch⁵, Germaine Minoungou⁶, Francois Thiaucourt⁷, Adama Diallo^{1,7}




Multiplex assays

- Rapid nucleic acid extraction and high-speed RT-qPCR in < 60 min
- Multiplex: PPRV, Foot-and-mouth disease virus, Parapoxvirus ovis, Goatpox virus, and Mycoplasma capricolum subsp. capripneumoniae



Article

"FASTCHECK^{FLI} PPR-like"—A Molecular Tool for the Fast Genome Detection of PPRV and Differential Diagnostic Pathogens

Sabrina Halecker¹, Thomas C. Mettenleiter², Martin Beer¹ and Bernd Hoffmann^{1,*} 

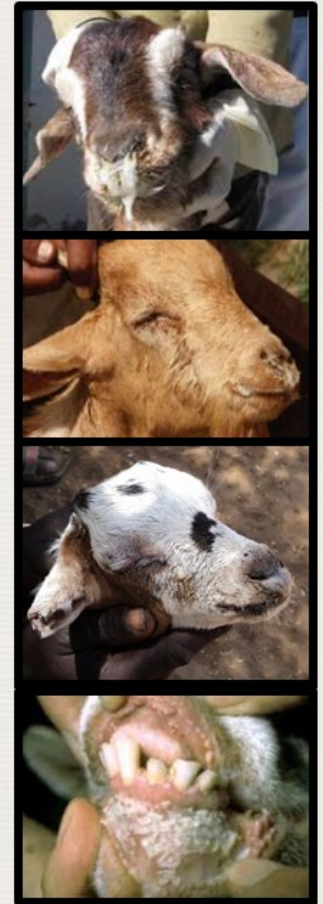


Joint FAO/IAEA Programme
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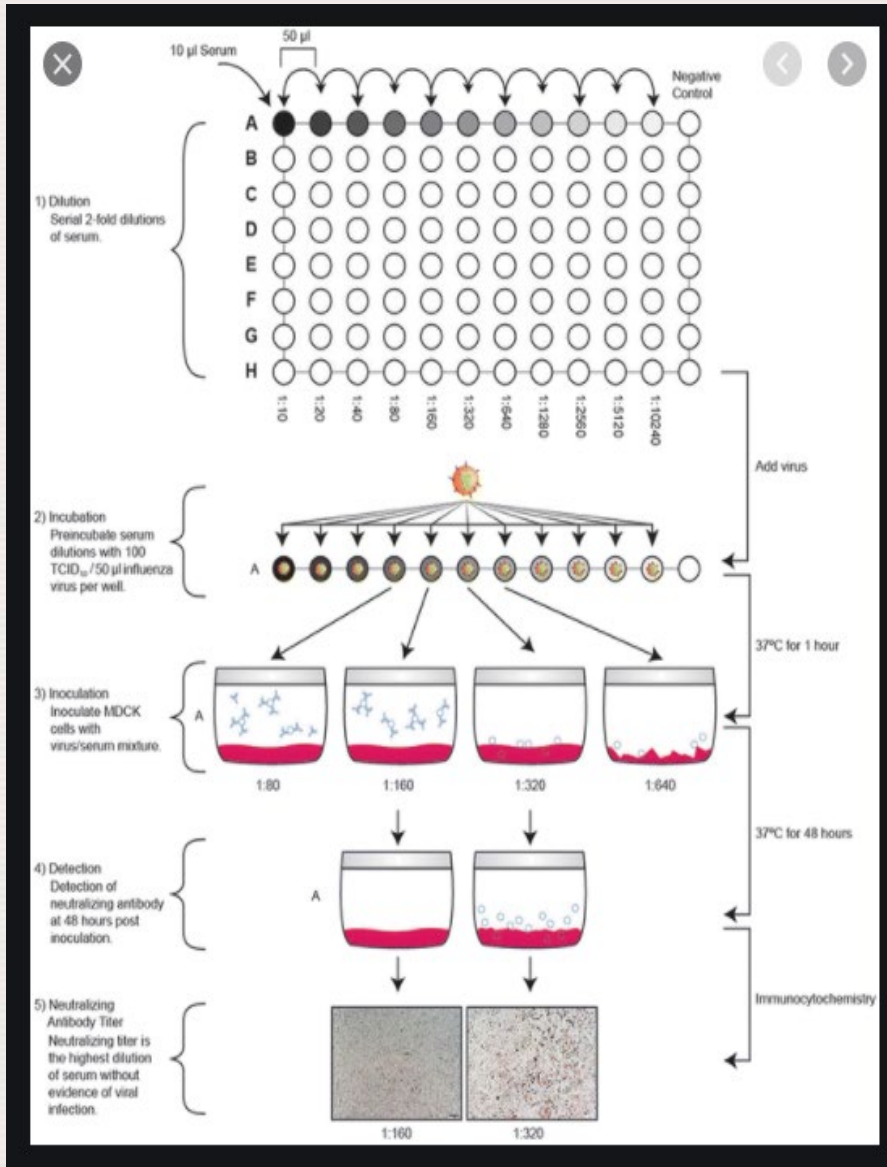
Serology

Serological

- Virus Neutralization
- Enzyme-linked immunosorbent assay (ELISA)
- Luciferase Immuno Precipitation System (LIPS)
- Samples required: clotted blood



Virus Neutralization



- Detects neutralising antibodies against the antigen - **gold standard**

Limitations

- require BSL-3 facilities in non-endemic countries
- Time consuming and technically complex

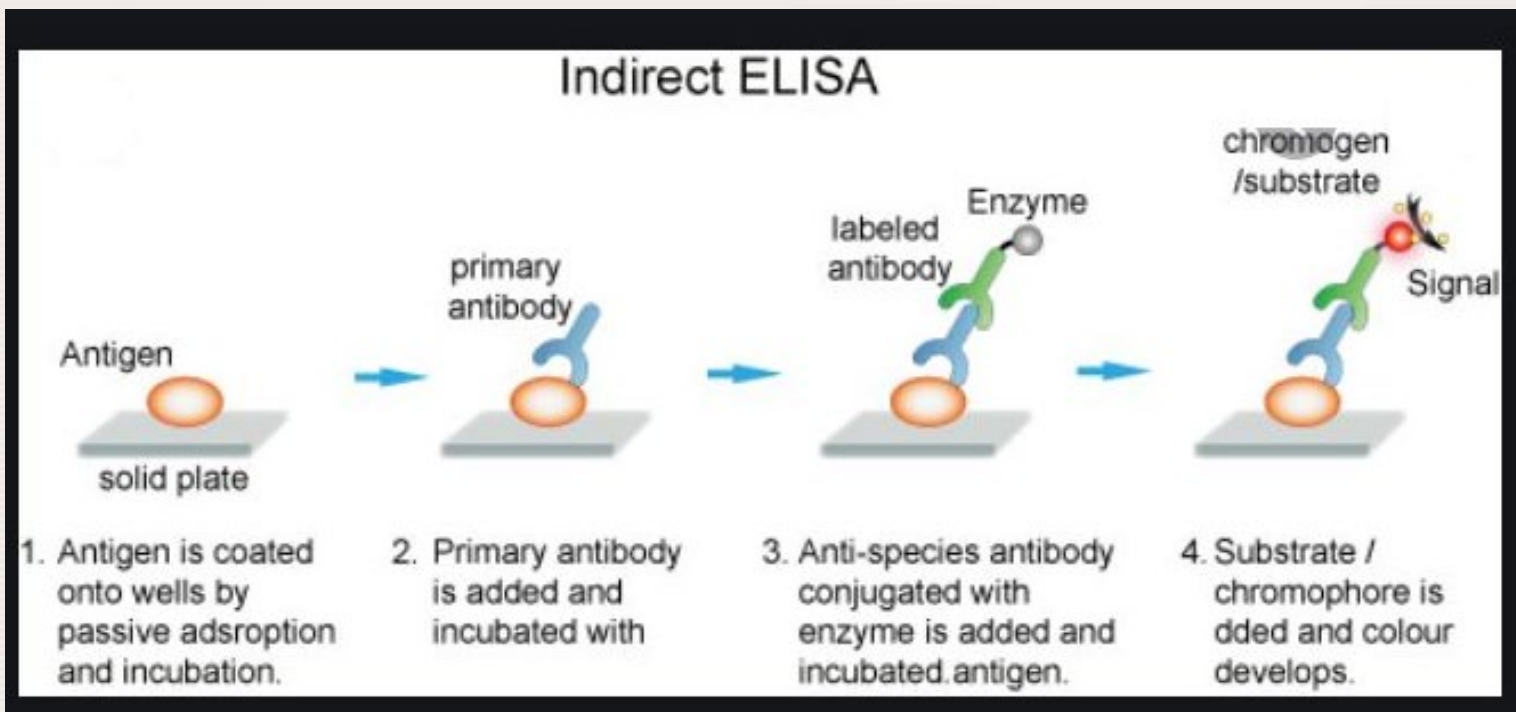
ELISA

Many choices available (Indirect and Competitive)

- Balamurugan V et al Development of an indirect ELISA for the detection of antibodies against Peste-des-petits-ruminants virus in small ruminants. Vet Res Commun. 2007 Apr;31(3):355-64. **100% specificity and 80% sensitivity.**
- Libeau G, et al Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. Res Vet Sci. 1995 Jan;58(1):50-5. **94.5 % sensitivity and 99.4% specificity**
- Singh RP, et al. Development of a monoclonal antibody based competitive-ELISA for detection and titration of antibodies to peste des petits ruminants (PPR) virus. Vet Microbiol. 2004 Jan 14;98(1):3-15 **98.4% sensitivity and 92.4% specificity**
- Bodjo SC, et al . Development and validation of an epitope-blocking ELISA using an anti-haemagglutinin monoclonal antibody for specific detection of antibodies in sheep and goat sera directed against peste des petits ruminants virus. Arch Virol. 2018 Jul;163(7):1745-1756. **93.4% sensitivity and 100% specificity**
- Choi KS et al. Rapid competitive enzyme-linked immunosorbent assay for detection of antibodies to peste des petits ruminants virus. Clin Diagn Lab Immunol. 2005 Apr;12(4):542-7. **93.4% sensitivity and 98.5% specificity**

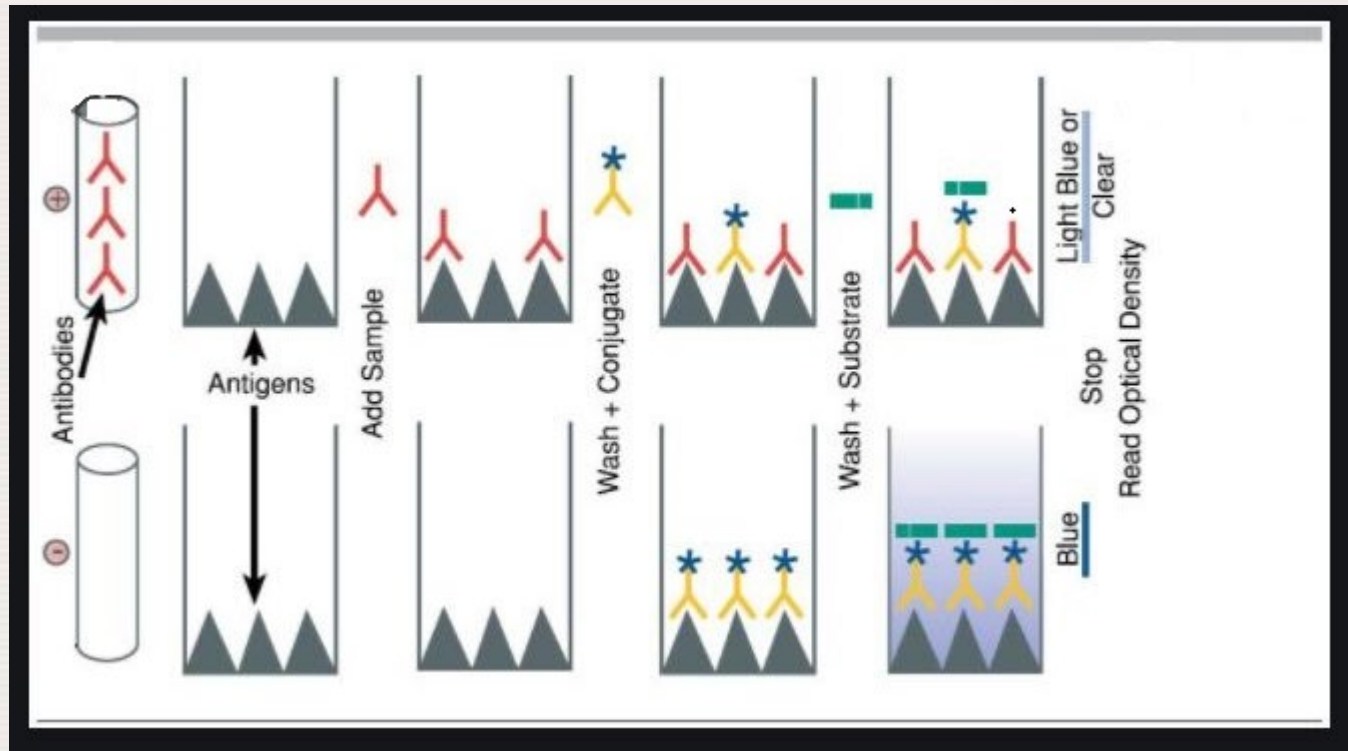
ELISA

- **Indirect**: species specific secondary antibody

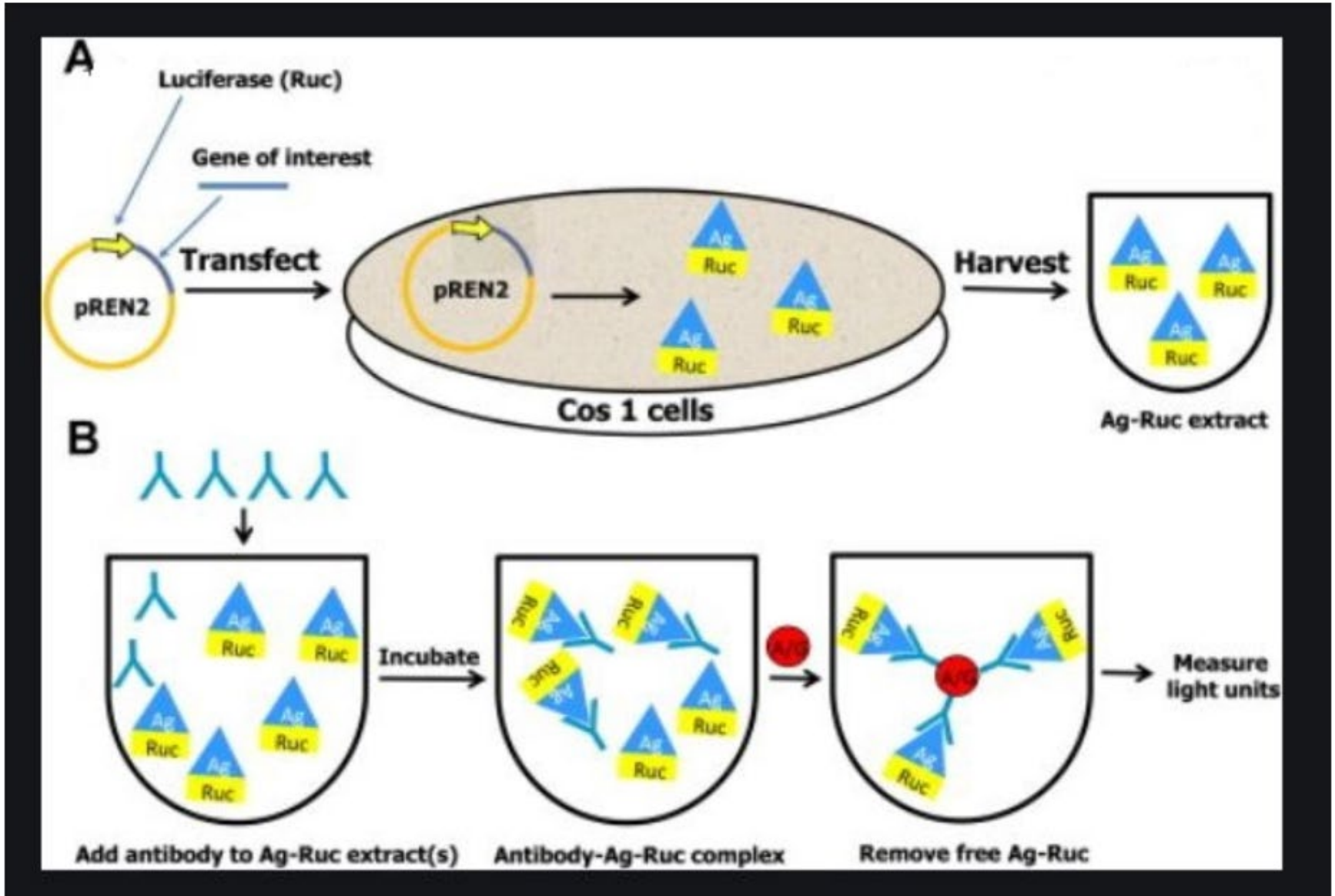


ELISA

- **Competitive**: Can be used for multiple species although not validated in wildlife yet.



Luciferase Immune Precipitation System (LIPS)



In this study, we have developed a Luciferase Immunoprecipitation System (LIPS) for the rapid detection of antibodies against PPRV in serum samples and for specific differentiation from antibodies against RPV. PPR and rinderpest (RP) serum samples were assayed by PPR-LIPS and two commercially available PPR-ELISA tests. The PPR-LIPS showed high sensitivity and specificity for the samples tested and showed no cross reactivity with RPV unlike the commercial PPR-ELISA tests which did cross react with RPV. Based on the results shown in this study, PPR-LIPS is presented as a good candidate for the specific serosurveillance of PPR.

Field tests

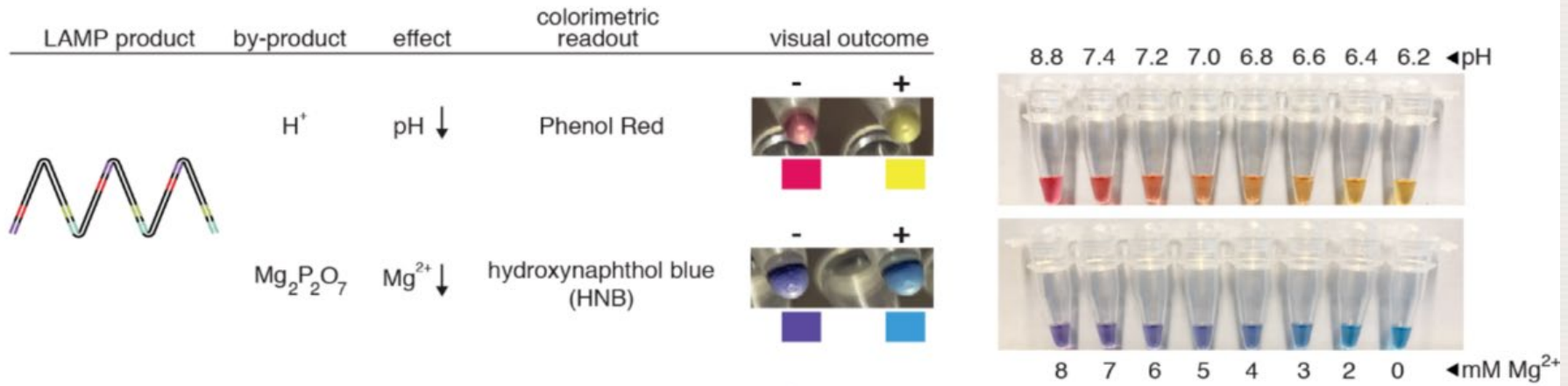
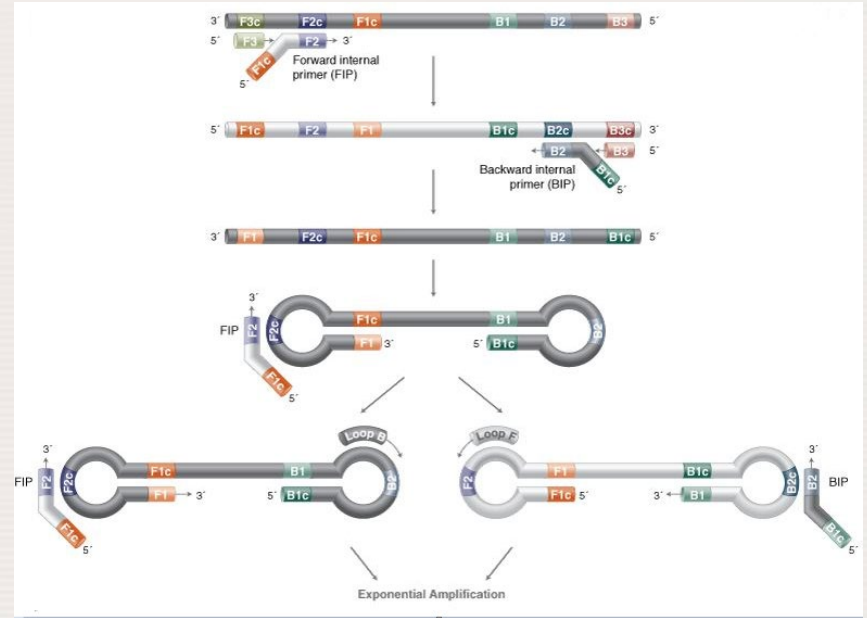
Pen-side/field tests

- RT-LAMP
- Lateral Flow Devices (LFD)
- MiniION

RT-LAMP

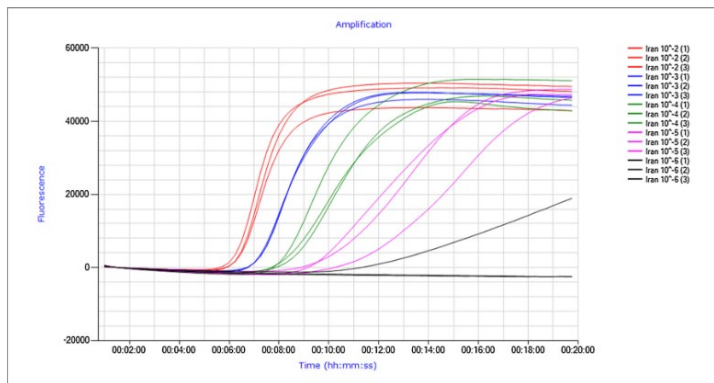
- Reverse transcription loop-mediated isothermal amplification (RT-LAMP)

No thermocycling like PCR
- constant temperature
between 60 and 65 °C



RT-LAMP

- 97% sensitivity : 100% specificity
- Comparable to RT-qPCR
- Could detect PPRV at 4 days post infection!



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journal homepage: www.elsevier.com/locate/jviromet

A rapid RT-LAMP assay for the detection of all four lineages of Peste des Petits Ruminants Virus

Paulina Rajko-Nenow*, John Flannery, Hannah Arnold, Emma L.A. Howson, Karin Darpel, Anna Stedman, Amanda Corla, Carrie Batten



RT-LAMP

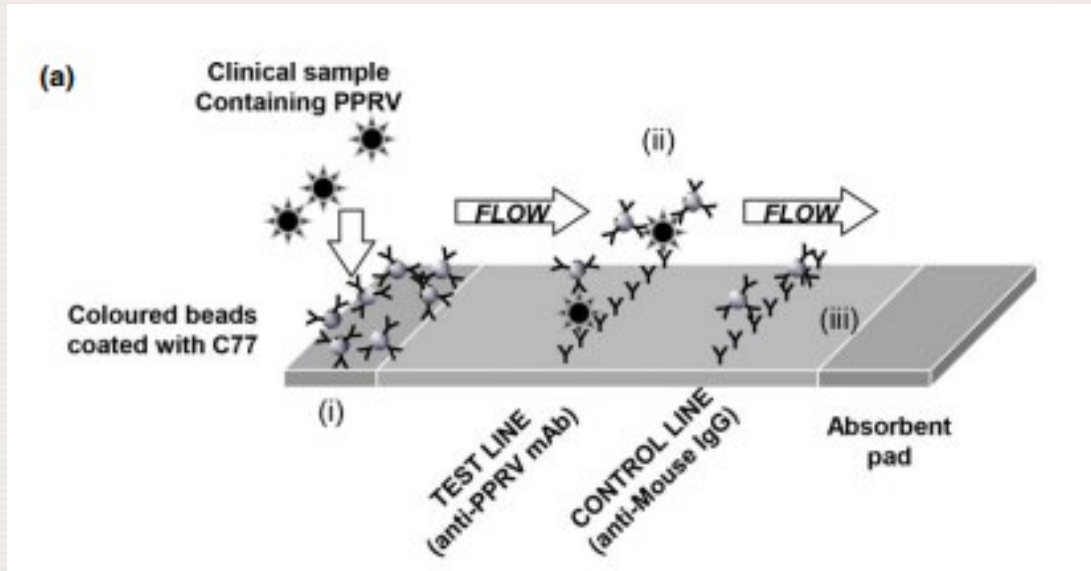
See also

Li L, et al Rapid detection of peste des petits ruminants virus by a reverse transcription loop-mediated isothermal amplification assay. J Virol Methods. 2010 Dec;170(1-2):37-41.

Mahapatra M, et al Detection of Peste des Petits Ruminants Virus (PPRV) Nucleic Acid Using a Novel Low-Cost Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) Assay for Future Use in Nascent PPR Eradication Programme. Viruses. 2019 Jul 31;11(8):699.

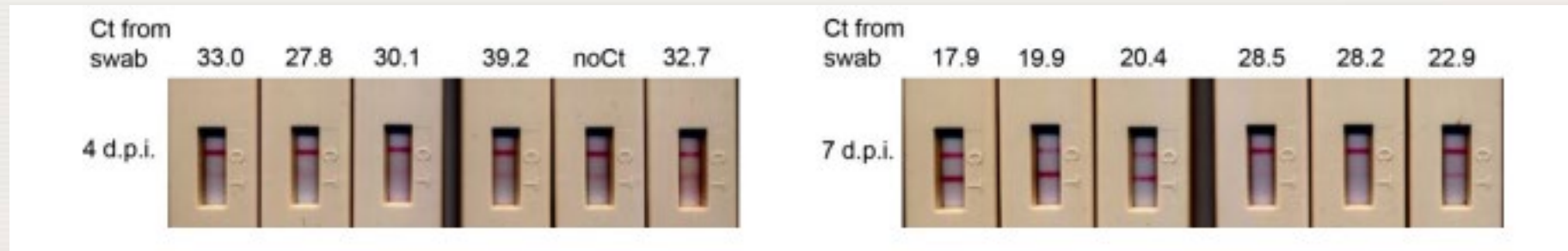
Niamat et al 2018 Loop-Mediated Isothermal Amplification Assay for Peste des Petits Ruminants Virus Detection and its Comparison with RT-PCR. Pak. Vet J. DOI: 10.29261/pakvetj/2018.106

Lateral Flow Device (LFD)



Monoclonal antibody C77 recognizes the PPRV H protein

Baron J, et al. Development and testing of a field diagnostic assay for peste des petits ruminants virus. *Transbound Emerg Dis*. 2014 Oct;61(5):390-6. **PESTE-test**



LFD

See also

Yang Y, et al. Development of real-time and lateral flow strip reverse transcription recombinase polymerase Amplification assays for rapid detection of peste des petits ruminants virus. *Virologia J.* 2017 Feb 7;14(1):24.

Cheng S, et al A new immunoassay of serum antibodies against Peste des petits ruminants virus using quantum dots and a lateral-flow test strip. *Anal Bioanal Chem.* 2017 Jan;409(1):133-141

Antigen Capture/LFDs

Three commercial antigen capture kits evaluated

- Antigen capture ELISA (ID-Vet)
- Two LFDs (PESTE-test and ID-Rapid PPR antigen)
- Worked better with Nasal > Ocular > Fecal

Received: 22 December 2019 | Revised: 15 May 2020 | Accepted: 28 May 2020



DOI: 10.1111/tbed.13660

ORIGINAL ARTICLE

Transboundary and Emerging Diseases

WILEY

Comparative evaluation of different antigen detection methods for the detection of peste des petits ruminants virus

Sabrina Halecker¹ | Sunitha Joseph² | Rubeena Mohammed² | Ulrich Wernery² |
Thomas C. Mettenleiter³ | Martin Beer¹  | Bernd Hoffmann¹ 

Commercial LFDs



➤ ID Rapid® PPR Antigen



TABLE 3 Comparative evaluation of various rapid detection methods for SRMV with regard to the suitability as a pen-side test

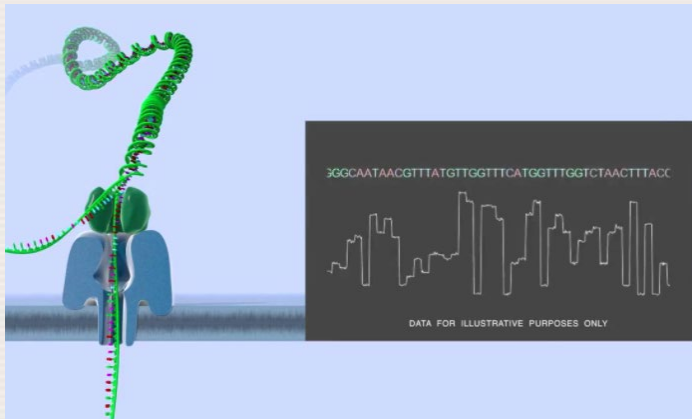
	Lateral Flow Devices		Antigen ELISA
	ID Rapid [®] PPR antigen	PESTE-test	ID Screen [®] PPR antigen capture
Handling of the test in general			
Simplicity	++	+++	+ ^a
Good runnability of the test strips	+++	+	n.a.
Duration (without preparation time)	30 min	30 min	2 hr
Supplied materials			
Simple instructions for use	++	+++	+ ^a
Suitability of the swabs	+++	+	n.a.
Additional equipment necessary	No	No	Yes
Preparation of reagents required			
Reagents/device should be brought to ambient temperature	No	Yes	Yes
Reagents have to be mixed in advance	No	No	Yes
Storage of the kit			
Room temperature (18°C–30°C)	+++	–	–
Refrigerator temperature (2°C–8°C)	+	+++	+++
Results			
Sensitivity	75.0%	53.3%	75.0%
No doubtful results	++	+	+++
Cut-off (in Cq values)	25–26	24–26	24–26
Test system applicable for differential diagnosis	No	No	No

Note: +++, completely agree; ++, rather agree; +, is insufficient; –, strongly disagree; n.a., not applicable.

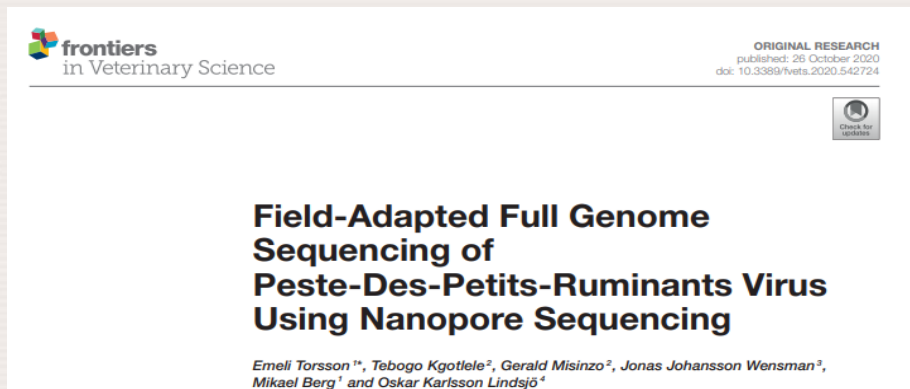
^aFor experienced users only.

Oxford nanopore MinION

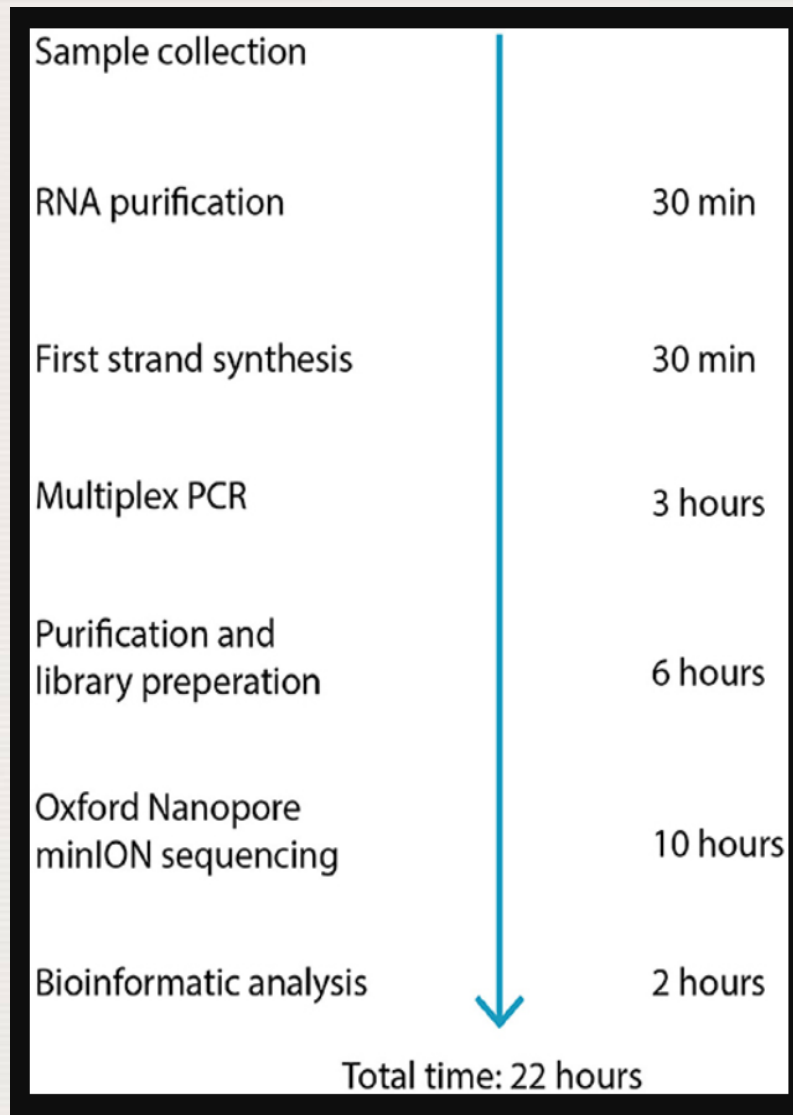
- Portable, real-time device for DNA or RNA sequencing
- Long reads, < \$1000
- Characteristic disruption in current as the analyte (DNA or RNA) passes through a nanopore.



Oxford nanopore MinION



- Field isolates from tissue samples of naturally infected goats sequenced (Tanzania)
- Full genome possible within 24 h of sampling





(Virtual Event) International Symposium on Sustainable Animal Production and Health – Current Status and Way Forward

28 June–2 July 2021, Vienna, Austria

[https://www.iaea.org/
events/aphs2021](https://www.iaea.org/events/aphs2021)



THANK YOU
Questions ?

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