Diagnostics available for African Swine Fever virus

Dr John Flannery

Regional workshop on swine disease diagnosis

30th October 2019

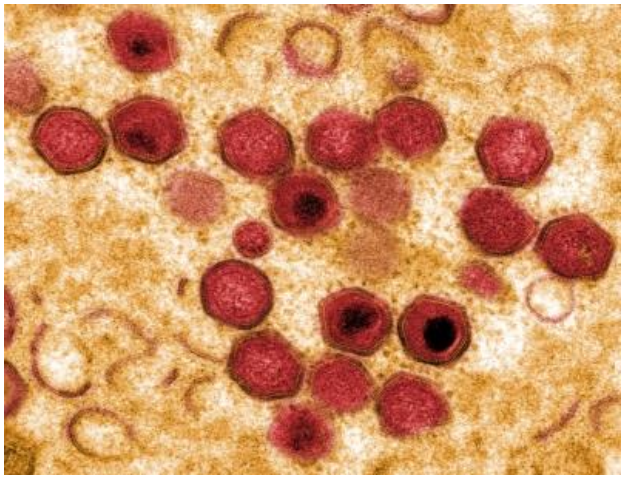
Beijing, Peoples Republic of China

BBSRC bioscience for the future

The Pirbright Institute receives strategic funding from BBSRC.

Overview

- The Pirbright Institute
  - Reference laboratories based at Pirbright
- Sample matrices
- Diagnostic tools to combat ASFV
  - Antibody detection tests
  - Virus isolation
  - Antigen detection tests
  - Molecular tests
  - Field-based or penside tests
- Summary of diagnostic techniques



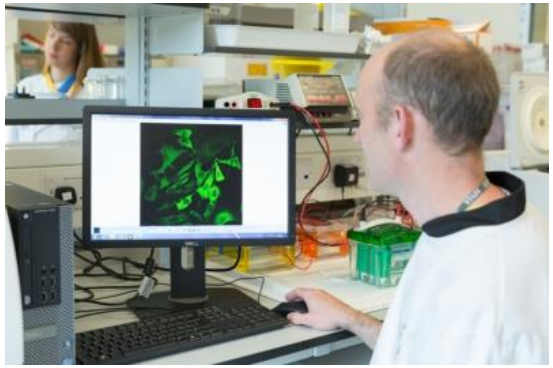
The Pirbright Institute

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 (354 million CNY)
- 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

Reference laboratories at Pirbright



Vesicular disease reference laboratories

Non-vesicular disease reference laboratories

ASF sample matrices and tests

- Whole blood
  - alive/dead pigs
- Lymph nodes
  - submandibular,
  - Inguinal
  - gastrohepatic
- Spleen
- Lung
- Kidney
- Tonsils
- Bone marrow
- Tonsil scrapings/ swabs

Serological analyses

- ELISA
- Lateral flow device (antibody)
- Immunoblotting test
- Indirect fluorescent antibody test
- Immunoperoxidase test

Virological & molecular analyses

- Antigen ELISA
- Lateral flow device (antigen)
- Virus isolation and HAD
- Direct fluorescent antibody test
- PCR and real-time PCR
- Sequencing

ASFV serological tests

ASFV antibodies can be detected from 12 days to 5 years post infection

- OIE indirect ELISA
- Commercial antibody ELISA

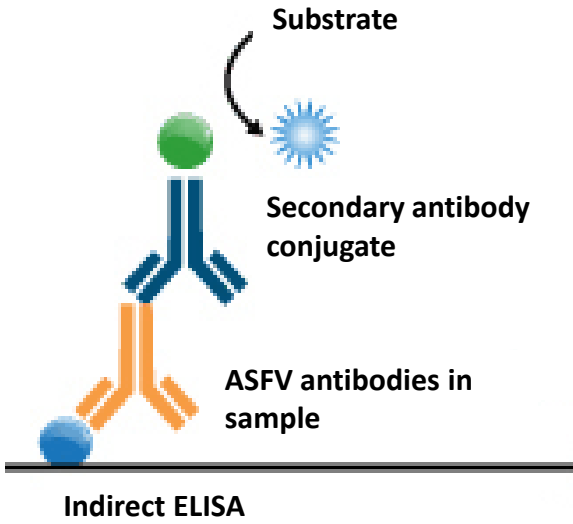
Screening assays

- Immunoblotting test (IBT)
- Indirect fluorescent antibody test (IFAT)
- Indirect immunoperoxidase test (IPT)

Confirmatory assays

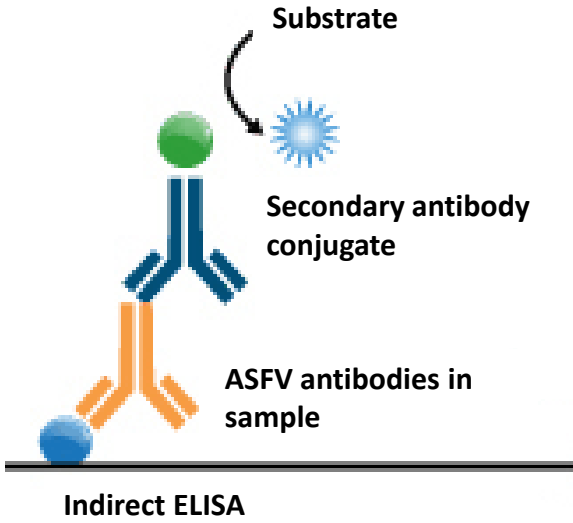
Indirect ELISA- OIE manual

- The OIE indirect ELISA (Pastor et al., 1990) uses cytoplasm soluble antigen (ASFV Spanish strain E70 grown in MS cells)
- Antigen coated onto wells and sample serum added
- Samples with ASFV antibodies against ASF will form an antigen-antibody complex
- Addition of conjugate, washing and addition of substrate will visualise positive samples



Indirect ELISA- OIE manual

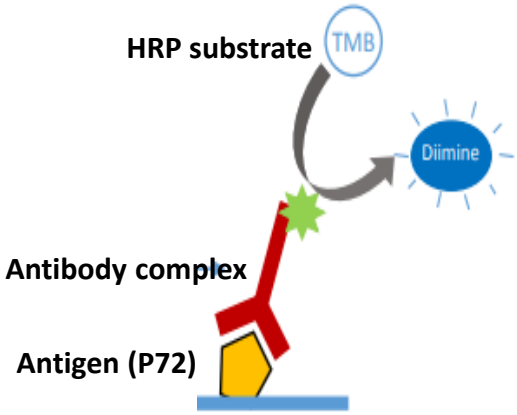
- OIE indirect ELISA test for ASF has high specificity (95.8%) and sensitivity (97.3%) (Gallardo *et al*., 2013)
    - *to allow confident diagnosis of ASF independent of the viral genotypes circulating in a particular region.*
- However, reagents are not commercially available but can be requested from OIE reference laboratory, CISA-INIA, Spain



Commercially-available antibody-detection ELISAs

- **Commercial ELISAs are available worldwide**

- INGEZIM PPA COMPAC K3 (INGENASA)→ blocking ELISA which uses a monoclonal antibody (MAb) specific of VP72 ASFV protein
- SVANOVIR® ASFV-Ab → indirect ELISA based on a recombinant antigen - the p30 (screening format)
- ID Screen® ASF → Competition ELISA kit for the detection of antibodies against P32 ASFV protein
- ID Screen® ASF → Indirect Multi-antigen indirect ELISA kit for the detection of antibodies against P32, P62 and P72 ASFV proteins

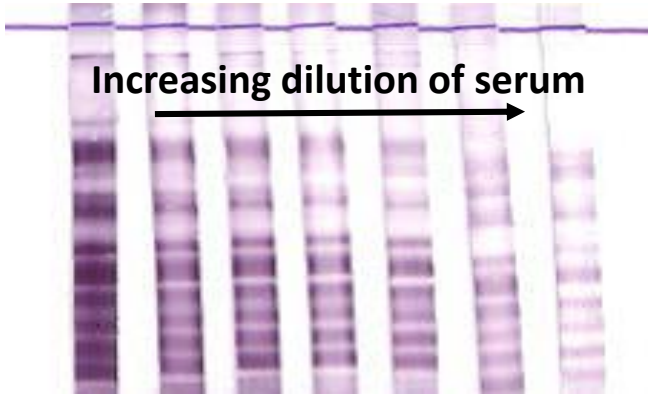


All kits have undergone validation and are used in European Union proficiency tests

Immunoblotting test (IBT)

- Described by Pastor et al, 1989 and Escribano *et al*., 1990
- Immunoblotting test (IBT) is a sensitive assay based on antigen-antibody recognition (Gallardo *et al*., 2013)
- ASFV viral proteins (electrophoretically separated using SDS-PAGE) are transferred to a nitrocellulose membrane.
- The membrane is then blocked using Tween/milk protein and cut into strips
- Serum is overlaid onto the antigen strip
- Specific antibodies against ASF are visualised by addition of an A-peroxidase conjugate protein and chloronaphtol as substrate

Courtesy: CISA-INIA, Spain

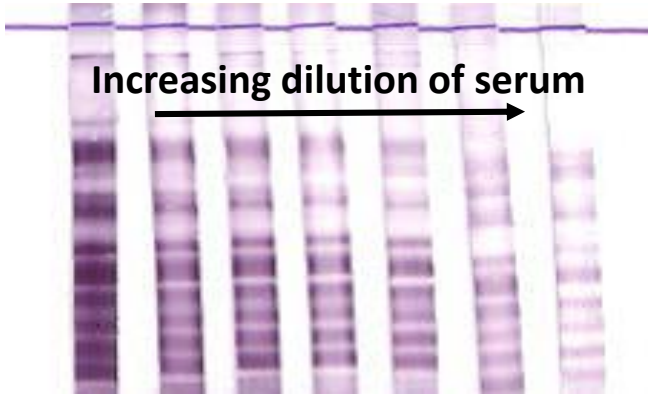


Immunoblotting test (IBT)

- Test takes ~3 hours to complete
- Reagents (strips) are not commercially available and must be requested from OIE reference laboratory, CISA-INIA, Spain

The IBT is the recommended test by the World Organization for Animal Health (OIE, 2012) in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals for the confirmation of positive and doubtful samples by ELISA

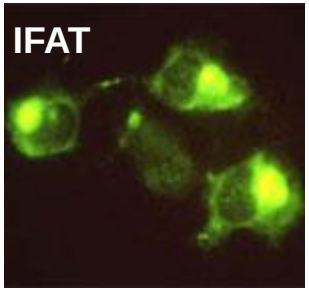
Courtesy: CISA-INIA, Spain

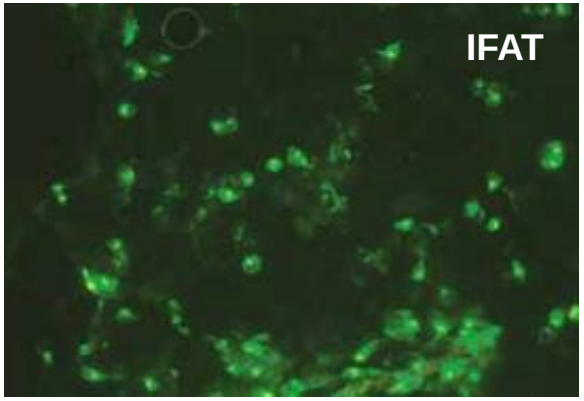


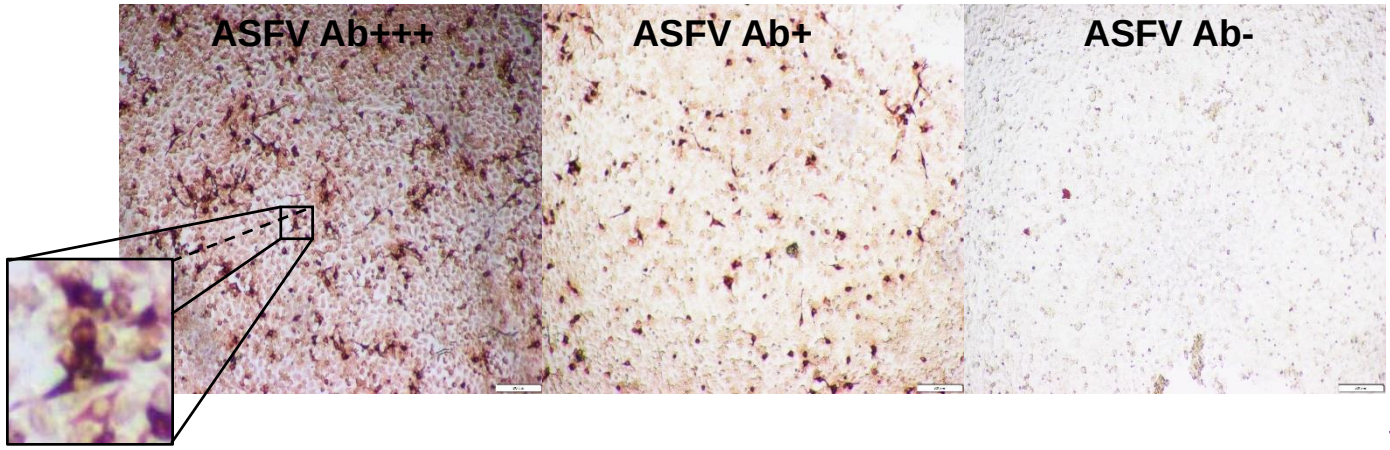
Indirect fluorescent antibody test (IFAT)
Indirect immunoperoxidase (IPT)

- Developed by Pan *et al*., 1974, Pan *et al*., 1982
- ASFV-infected Vero or MS cells are fixed and used as antigens
- Antibody-antigen complexes are visualised by the use of fluorescein isothiocyanate or peroxidase conjugated IgG
- IPT considered easier to interpret results and uses simpler equipment

IFAT is used as a confirmatory test (areas free of ASFV but with positive/inconclusive ELISA results)

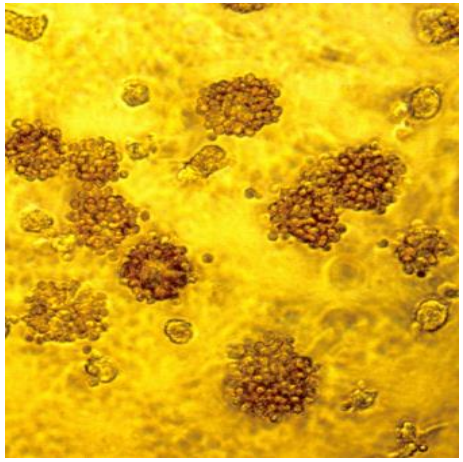


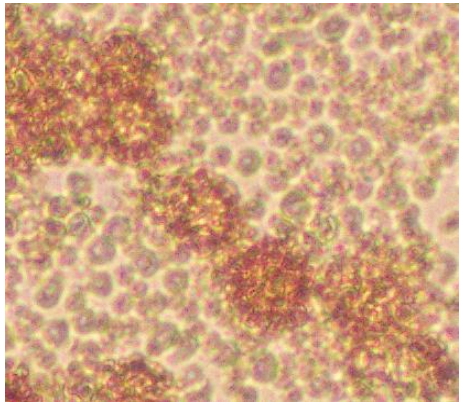


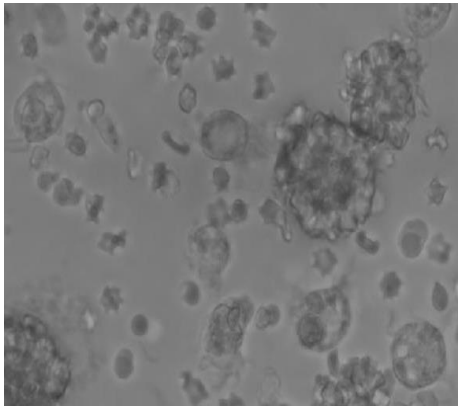


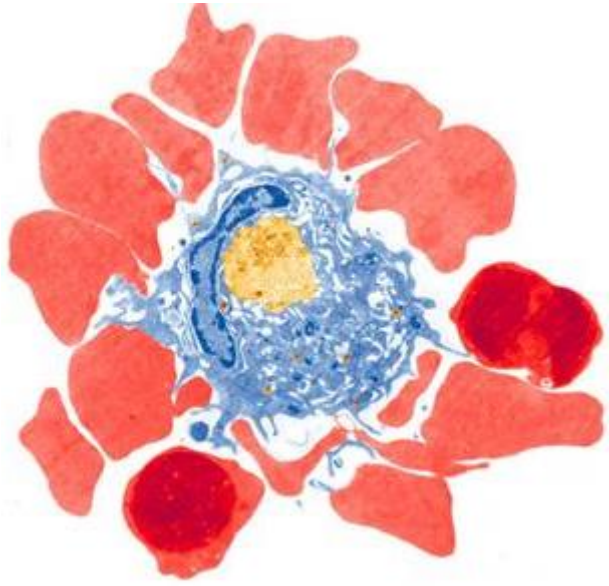
Virus isolation and HAD

- ASFV infects and replicates in primary leukocyte cultures from pig peripheral blood (Malmquist and Hay, 1960)
- Inoculation of sample material on susceptible primary cell cultures of porcine origin, monocytes and macrophages cells
- ASFV replicates in the cells and the CPE (haemadsorption and cell lysis) will be produced in the infected cells after 48 hours
- Rosettes form due to haemadsorption (HAD) of porcine erythrocytes on ASFV infected macrophages



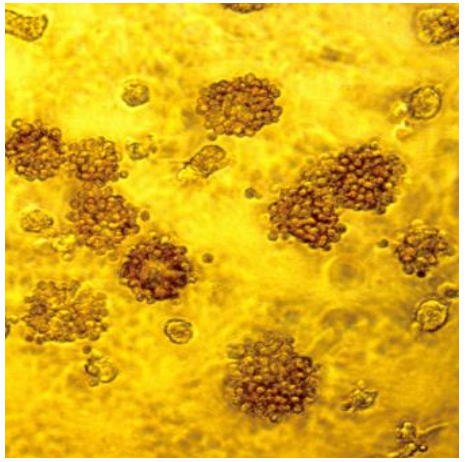




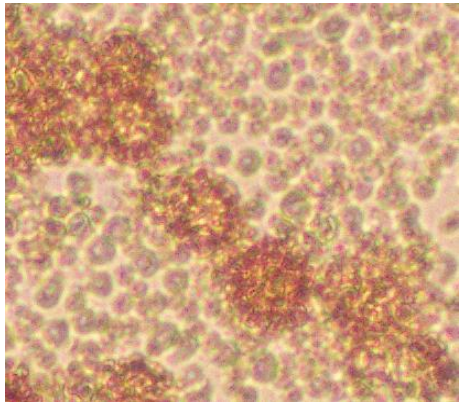


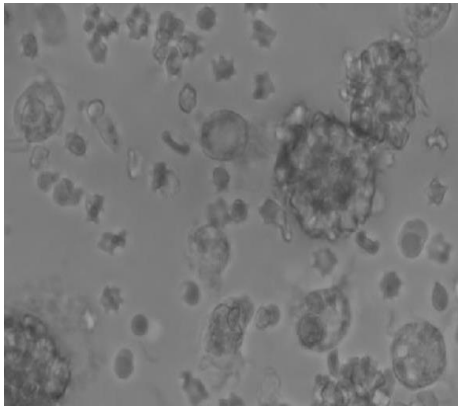
Virus isolation and HAD

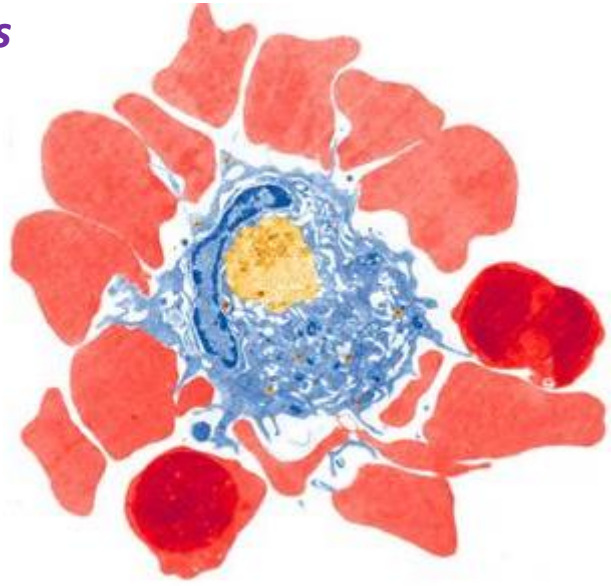
- **Test is highly specific:** only ASFV is capable of haemadsorbing in leukocyte cultures
- **Primary cell culture is required**
- Not all ASFV strains haemadsorb
- Test duration of 3- 10 days



Virus isolation and identification by HAD are recommended as a reference test for the confirmation of positive results

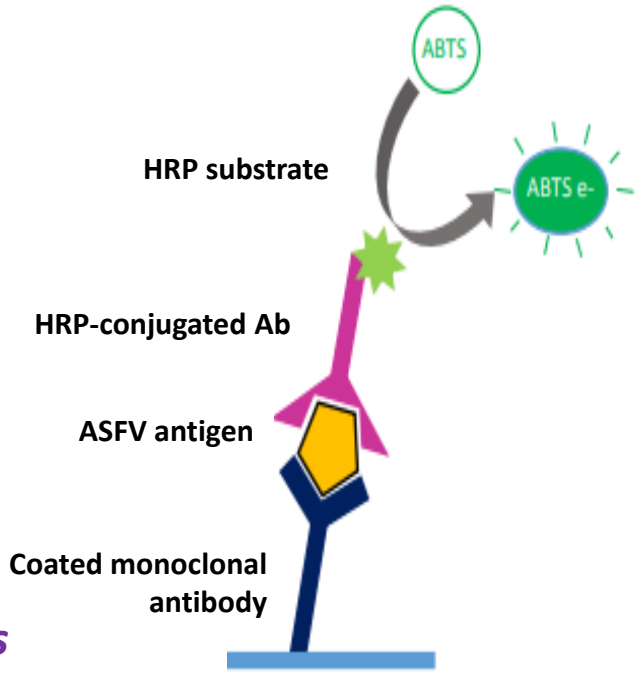






Antigen detection by ELISA

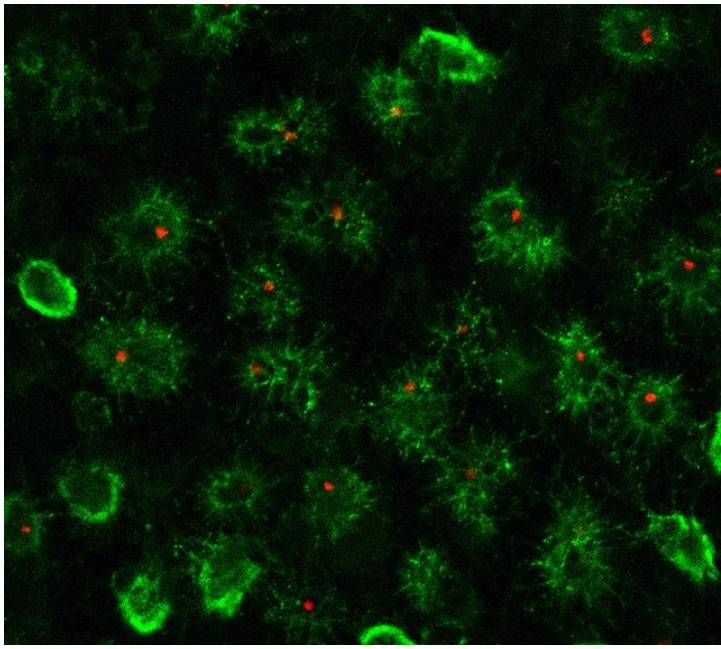
- Viral antigens can also be detected using ELISA, but it is only recommended for acute forms of disease
- Monoclonal anti-VP72 antibody coated plates
- ASFV antigen in the sample will bind to the specific anti-VP72
- HRP-conjugated MAb specific for a different epitope of VP72 is added
- Colorimetric reaction after the addition of the substrate



The antigen ELISA has a poor sensitivity and is recommended to use the antigen ELISA only as a 'herd' tests and in conjunction with other virological tests

Direct fluorescent antibody test (FAT)

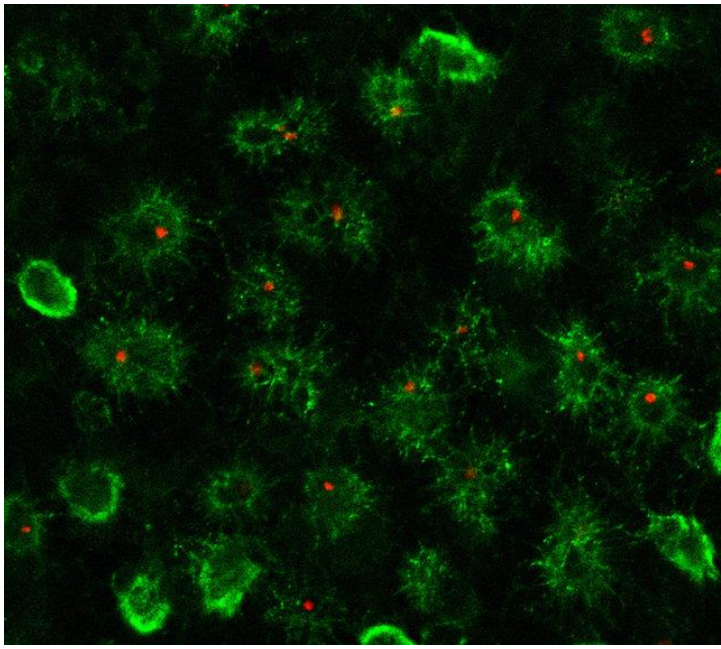
- Microscopic detection of viral antigens on impression smears or thin cryosections
- Intracellular antigens are detected using FITC-conjugated specific antibodies.
- Fluorescent inclusion bodies or granules appear in the cytoplasm of infected cells.
- Useful to detect non-haemadsorbing ASFV strains antigen in leucocyte cultures in which no HAD is observed



Courtesy: Dr Pippa Hawes, Pirbright

Direct fluorescent antibody test (FAT)

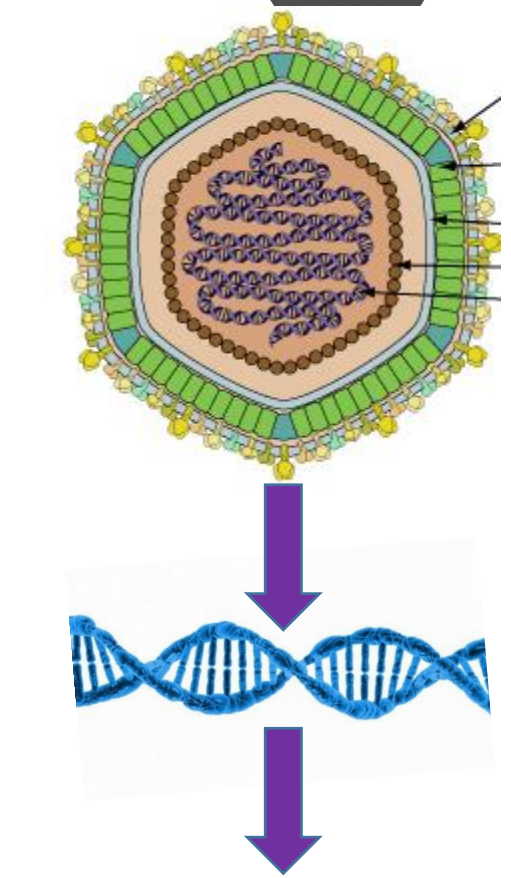
- Highly sensitive for peracute and acute ASFV infection
- Less sensitive for subacute and chronic disease due to the presence of antigen-antibody complexes
    - Host antibodies compete with the ASF-conjugated antibody

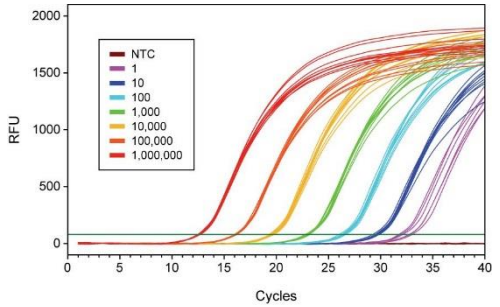


Courtesy: Dr Pippa Hawes, Pirbright

ASF PCR

- PCR can be directed to conserved genome regions to detect all ASFV isolates.
- Genome is stable and can be detected in many sample types even if infectious virus is inactivated
- PCR enables specific and sensitive detection with 5 hours (including DNA extraction)

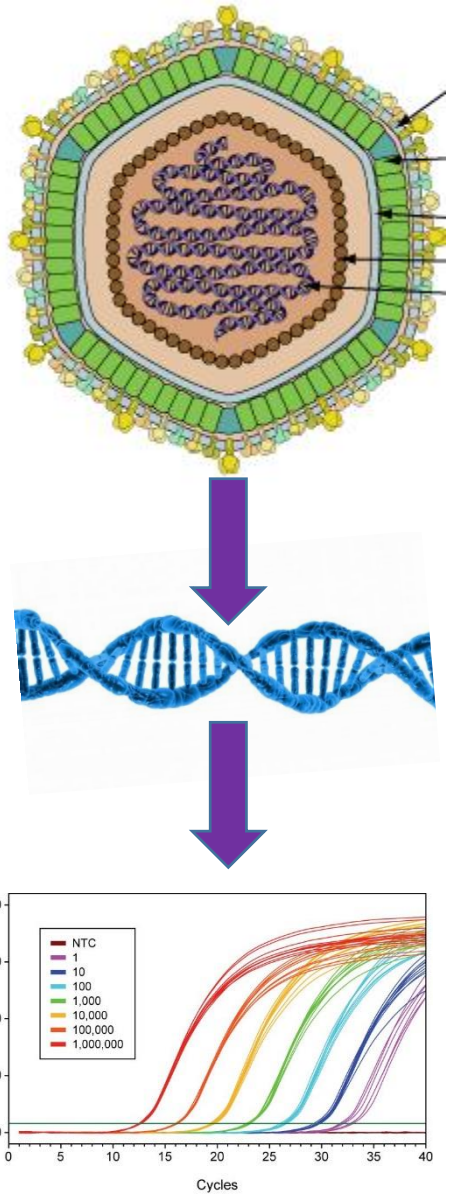




ASF PCR

- One conventional PCR (**Agüero *et al.*, 2003**) and 2 real-time PCRs in OIE Manual
- ASF/CSF duplex assay (**Haines *et al.*, 2013**)
- Commercial assays are now available (IDVet, ThermoFisher)
  - Additional assays being developed due to lucrative market

Real-time PCR assay	Target	IC	Analytical sensitivity	
King *et al.*, 2003	VP72	"Mimic"	10-100	OIE
Zsak *et al.*, 2005	VP72	None	1.4-8.4	
McKillen *et al.*, 2010	9GL	None	20	
Tignon *et al.*, 2011	VP72	β-Actin	5.7-57	
Fernandez Pinero *et al.*, 2012	VP72	β-Actin	18	OIE

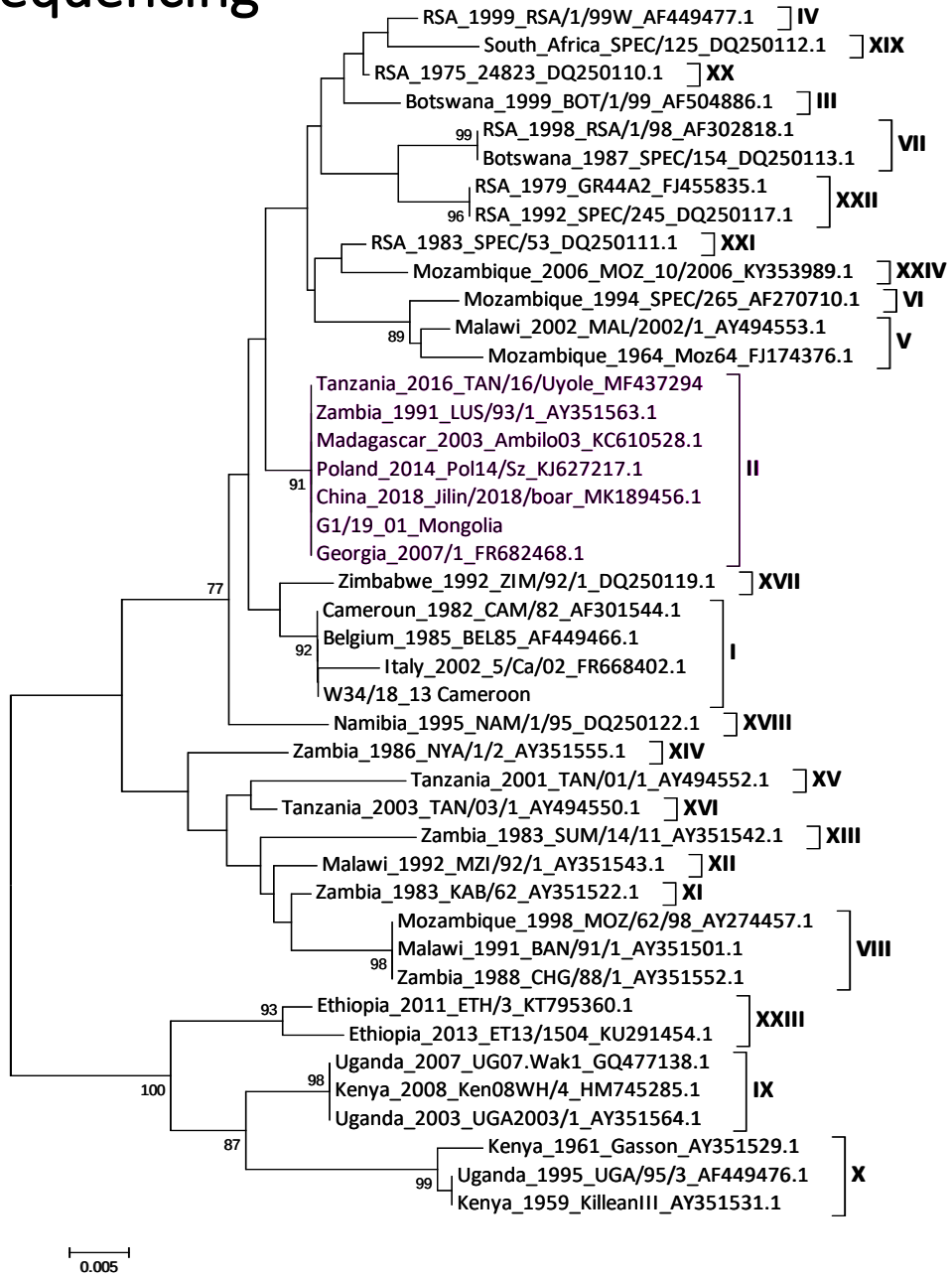


ASF Sequencing targets

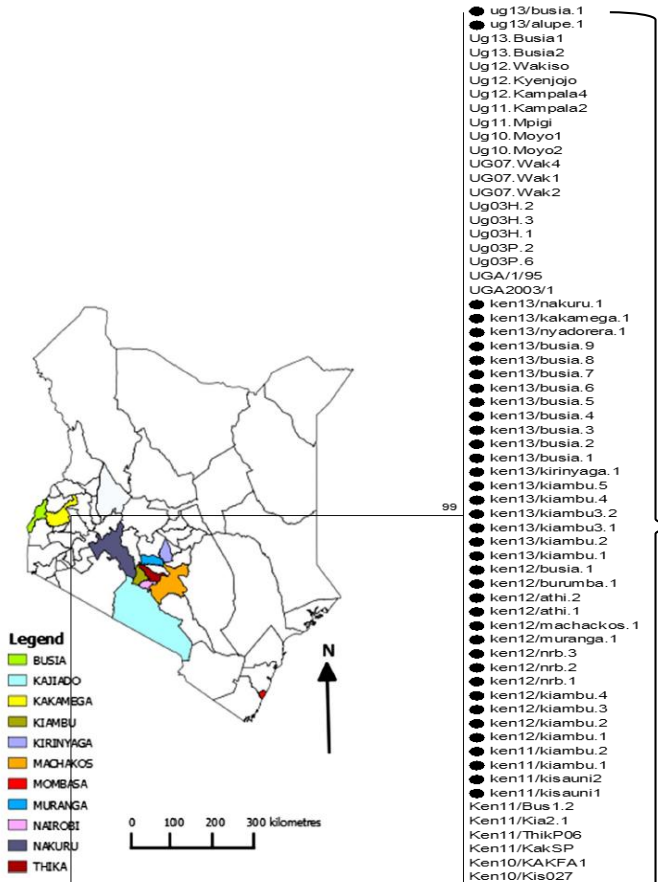
ASFV genotyping is based on the analysis of three independent regions located at the conserved central area of the ASFV genome comprising:

- Partial sequence of the C-terminal end of the *B646L* gene encoding VP72 (Bastos *et al*., 2003)
  - Classification of the ASFV genotypes (Boshoff *et al*., 2007)
- Full sequence of *E183L*-gene encoding the p54 protein
  - Additional epidemiological information of p72 genotype I viruses (Gallardo *et al*., 2009)
- Sequencing of the central variable region within *B602L*-gene (CVR) Determining the origin and helps map the spread of closely related ASFV strains (Nix *et al*., 2006; Gallardo *et al*., 2011)

P72 gene sequencing

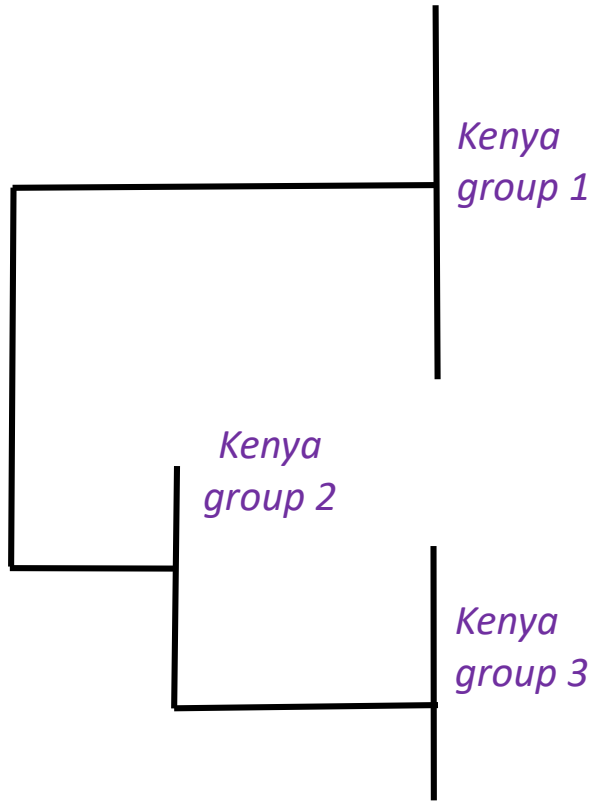


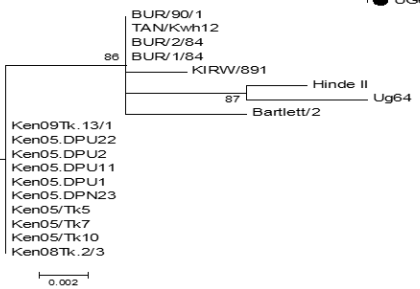
CVR gene sequencing provides higher resolution



Genotype IX

CVR sequencing



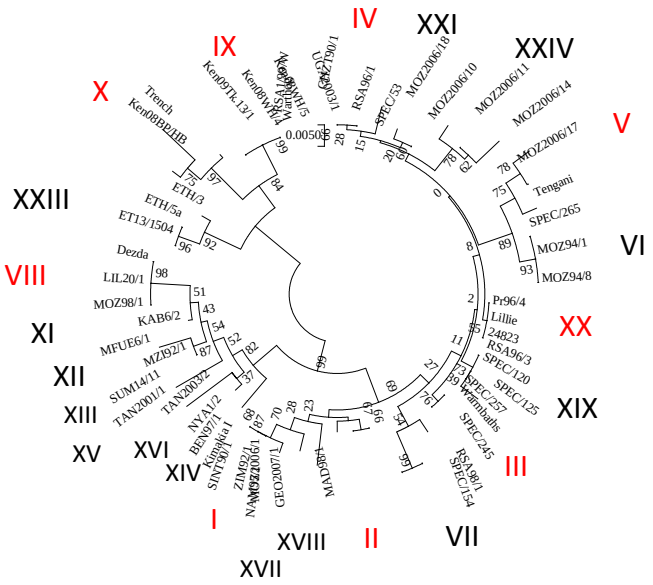


Genotype X

Multi-locus sequence typing of African swine fever viruses from endemic regions of Kenya and Eastern Uganda (2011–2013) reveals rapid *B602L* central variable region evolution

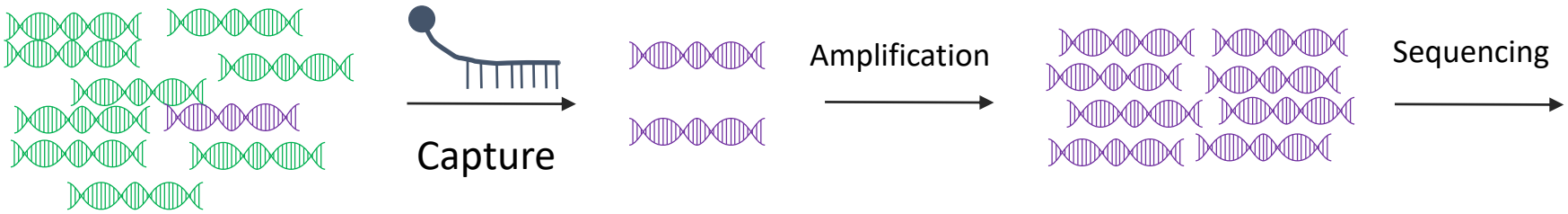
C. K. Onzere[1,4] · A. D. Bastos[2] · E. A. Okoth[1] · J. K. Lichoti[3] · E. N. Bochere[1] · M. G. Owido[1] · G. Ndambuki[1] · M. Bronsvoort[4] · R. P. Bishop[1]

Whole genome sequencing



- Whole genomes only available for selected ASFV genotypes
  - Mostly Eurasian and East African strains
- Technically challenging due to contamination with host genome – low read numbers for viral DNA
- Repetitive sequences at termini mean Illumina reads could introduce errors, particularly for novel sequences
- Terminal inverted repeats very difficult to assemble.

Moving towards probe capture technologies to purify viral sequences from host



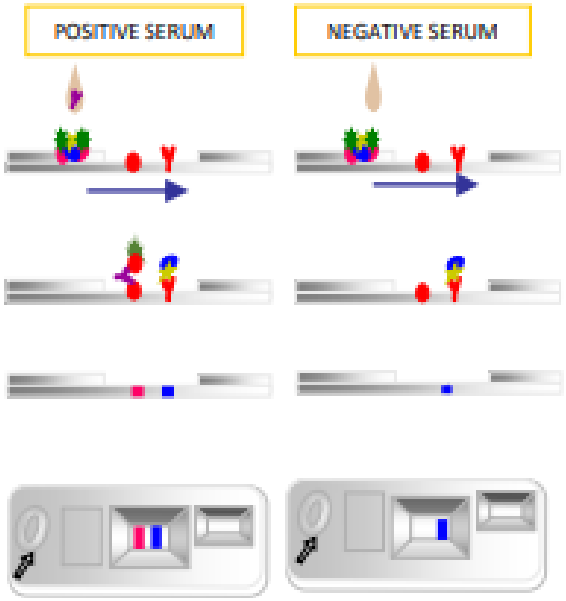
Courtesy: Dr Chris Netherton, Pirbright

ASFV field-based diagnostics

Penside test- Lateral flow device to detect ASFV antibodies

INgezim PPA CROM:

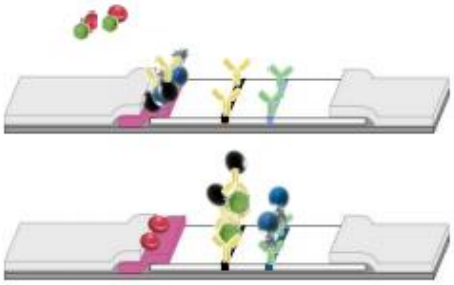
- Direct immunochromatography utilising ASFV VP72
  - VP72 and a control protein bound to coloured latex
- The assay is able to detect ASFV antibodies
  - 10 to 21 days post infection (*Cappai et al., 2017)*
  - High sensitivity: 99% with OIE ELISA
  - High specificity: 99.9% with OIE ELISA



LFDs are not considered suitable for confirmatory diagnosis

Penside test- Lateral flow device to detect ASFV antigen

INgezim ASF CROM Ag:



- Direct immunochromatography utilising ASFV VP72
  - VP72-specific MAb and a control protein MAb bound to coloured latex
- The assay is able to detect ASFV at high titres (C_T<30)
  - Similar sensitivity to Ag ELISA (*Sastre et al., 2016)*
  - Overall sensitivity: 76% with qPCR
  - High specificity: 99.6% with qPCR

LFDs are not considered suitable for confirmatory diagnosis

Summary of main diagnostic tests for ASF

Comparison of available tests

Serological detection

Assay	Time	Sensitivity	Specificity	Cost	Comments
ELISA	3 hours	+	+	$	Screening test
Immunoblotting	3 hours	++	++	$$$	Confirmatory test
Indirect FAT/ IPT	4 hours	+++	+	$$$	Confirmatory test

Virus detection/characterisation

Assay	Time	Sensitivity	Specificity	Cost	Comments
Antigen ELISA	3 hours	+	++	$	Screening test
PCR	5 hours	+++	+++	$$	Most commonly used assay
HAD	3-10 days	++	+++	$$$	Used in reference laboratories
Direct FAT	75 min	+++	+++	$$$	Confirmatory test
Partial sequence analysis	3 days	n.a.	n.a.	$$$	Genotype determination Can be applied in most laboratories
Full-genome sequence analysis	Weeks-months	n.a.	n.a.	$$$$$	Used in reference laboratories and involves complex bioinformatics

Interpretation of diagnostic test results

Sample	Ab ELISA	Conclusion	PCR CT	Conclusion	Diagnostic conclusion

- Limited diagnostic capabilities limit successful interpretation of results
- Suitable for regional laboratories
- Multiple tests are needed for complete diagnostic conclusion

Interpretation of diagnostic test results

	Serological tests			Virological & molecular tests					
Sample	Ab ELISA	IBT	IFAT/ IPT	Ag ELISA	PCR 1 CT	PCR 2 CT	CPE	HAD	FAT	Diagnostic conclusion
1	-				+ 20					
2	-/+				-					
3	+				-					
4	+				+ 32					
5	-				+ 38					

- **Ab ELISA:** Antibody ELISA
- **Ag ELISA:** Antigen ELISA
- **IBT:** Immunoblotting test
- **FAT:** Fluorescent antibody test
- **CPE:** Cytopathic effect in cell culture
- **HAD:** Haemadsorption test
- **IFAT/IPT:** Indirect fluorescent antibody test/ Immunoperoxidase test
- **PCR:** Polymerase chain reaction assay
- **n.d.** test not performed

- Multiple tests should be performed
- Important to consider results of each test and the test limitations
- PCR can give rise to contamination if not properly controlled
- Ensure seamless communication with veterinary authorities/field vets to obtain additional samples
- Liaise with OIE reference laboratories for confirmation of results

OIE reference laboratory support

The OIE reference laboratory at Pirbright can help with

- Provision of reagents for ASFV and other viruses
- Interpretation of diagnostic results
- Support for ISO/IEC17025 accreditation
- Diagnostic kit evaluation
- Training in diagnostic tests at Pirbright

Please do not hesitate to contact us concerning any of the above

Pirbright Institute

Non Vesicular Reference laboratories

- Dr Carrie Batten (Head of laboratory)
- Dr Paulina Rajko-Nenow
- Lorraine Frost
- Dr Mark Henstock
- Dr Martin Ashby
- Dr Simon King
- Dr Amanda Corla
- Matthew Tully
- Katie Harris
- Rebecca Moore
- Laura Marsala
- Sarah Belgrave
- Julie Maryan

ASFV research groups at Pirbright

- Dr Linda Dixon (OIE expert ASFV)
- Dr Chris Netherton
- Dr Pip Beard (OIE expert Capripoxviruses)
- Dr Ana Reis
- Lynnette Goatley
- Dr Raquel Portugal

Department for Environment Food & Rural Affairs

BBSRC bioscience for the future

www.pirbright.ac.uk