STAR-IDAZ
International Research
Consortium on Animal Health

Roadmaps for African Swine Fever

- **Development of candidate vaccines**
- **Development of diagnostic tests**

SIRCAH Deliverable 3.3

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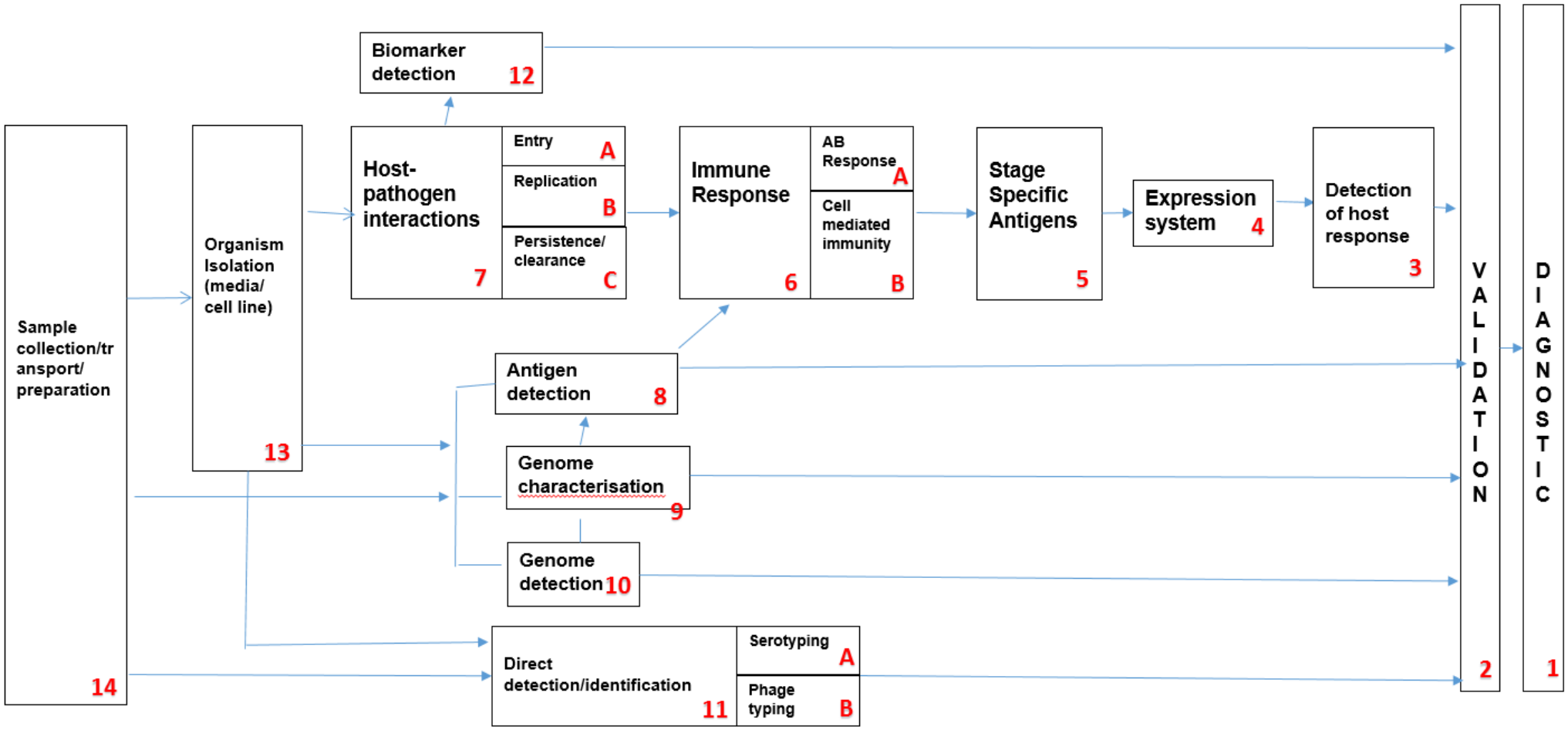
Roadmap for development of diagnostics tests for ASF



Lead summaries are in draft form until validated by the Global ASF Research Alliance (GARA)

ASF Diagnostics Test Development- Lead Summary 1

Title:	State-of-the-art ASF diagnostics

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Fit for purpose tests should be available or developed for the different purposes (surveillance, response, and recovery). The test systems should be reliable, easy to use, cost effective and should provide sufficient diagnostic sensitivity and specificity. Pen side tests would be helpful to enhance detection and improve response under circumstances which do not allow rapid laboratory testing. The test systems should cover both domestic and wild pigs. ASFV detection should also take tick testing into consideration.
Whole genome sequencing pipelines using NGS technology

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Tests needed for different purposes (both direct and indirect).
Sufficient diagnostic specificity and sensitivity.
Reliable internal controls for qPCR testing in suboptimal materials (e.g. from wild boar carcasses)
Further optimisation and development of pen-side tests and other field-deployable diagnostic tests (virus and antibody detection, sequencing)
Alternative sample types: e.g. easy sampling for wild boar
Detection of the same/different ASFV isolate in a single reaction.
Diagnostics in ticks.
Reduce costs.
Reference materials and gold standard assays.

Solution Routes

What approaches could/should be taken to address the research question?

Develop new technologies (e.g. not requiring permission to work with ASFV) which can bring more producers to the market.

Dependencies

What else needs to be done before we can solve this need?

Definition how to use the available diagnostics
Further field validation under different outbreak conditions.
Infrastructure and/or expertise for reliable diagnostic services in regional labs in endemic countries; trained personnel.
Intensify training and follow-up activities for international harmonisation of diagnostic tests.

State of the Art

Existing knowledge including successes and failures

A wide variety of reliable laboratory techniques fit-for-purpose are available either for ASF virus and antibody detection, and a strategic use of both is the recommended approach for detecting ASFV.
The most commonly used techniques for virus detection and identification are qPCR. Haemadsorption (HA), and direct immunofluorescence (DIF) are further techniques. Sequencing (including whole-genome sequencing with NGS) is used for genotyping.
The most common, practical and inexpensive techniques for ASF antibody detection is the ELISA and, as confirmatory tests, the immunoblotting assay (IB), Indirect immunofluorescence antibody test (IFA), and the Immunoperoxidase Test (IPT).

Projects

What activities are planned or underway?

Lead Summary 2

Title:	Validation of reliable tests, including pens-side, being “fit for purpose” for surveillance, response, and recovery

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Existing and novel diagnostics need to be validated in the field, according to different scenarios and different purposes (including surveillance). ELISA tests for detection of antibodies need validation in alternative sample types. Available pen-side test needs to be validated to enhance detection and improve surveillance also *e.g.* in wildlife in Africa.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Field validation should take into consideration global scenarios and the respective epidemiological contexts.
Validation in a wide range of alternative sample types.
Evaluation under experimental conditions of performance and overall accuracy of available ELISAs and PCR tests for surveillance.
Field validation of commercial confirmatory serological tests.
Standardisation and validation of ELISA tests to detect antibodies against *Ornithodoros* tick saliva antigens in bitten animals.
Reduction of costs.
Easiness to use (from the point of view ISO 17025 accreditation).

Solution Routes

What approaches could/should be taken to address the research question?

Dependencies

What else needs to be done before we can solve this need?

Reference materials
Ring tests
Training measures

State of the Art

Existing knowledge including successes and failures

Test systems for most questions available.
Validation studies are ongoing on several ASF diagnostics, including new ELISAs for antibody detection and pen-side ones (*e.g*. lateral flow devices, LFD).

Projects

What activities are planned or underway?

Lead Summary 3

Title:	Develop diagnostic indirect tests for the detection of the disease

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The development of diagnostic tests able to reliably detect low titres of antibodies and of new assays based on cellular immunity for the detection of the disease.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Varying host immune response to the virus between both virus' isolates and host species.
Testing of bad quality wild boar sera.
Laboratory capacities (variability of results among laboratories).

Solution Routes

What approaches could/should be taken to address the research question?

Dependencies

What else needs to be done before we can solve this need?

State of the Art

Existing knowledge including successes and failures

Knowledge was gained and collated on suitable antigens and their expression for serodiagnosis of ASF. Five serological methods were recently tested, including three commercial ELISAs, the OIE-ELISA, and the confirmatory immunoperoxidase test (IPT).

Projects

What activities are planned or underway?

Lead Summary 6

Title:	Detection of immune response in ASFV infected animals

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
The clarification of the immune response mechanisms in infected animals, as to improve detection of infected animals.

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
Understanding the actual role of virus driven host immunomodulation in the process of virus infection in swine. Find specific host reactivity pattern.

Solution Routes *What approaches could/should be taken to address the research question?*
Determine patterns of activation of immunologically relevant host genes. Use of transcriptomics technology to identify specific host reactivity pattern.

Dependencies *What else needs to be done before we can solve this need?*
Improve understanding of host virus interaction at the level of the infected cells. Infection studies under BSL3AG

State of the Art *Existing knowledge including successes and failures*
Advances have been achieved in identifying and understanding the function of virus genes modulating the host response and its direct effect during the process of infection in the natural host.

Projects *What activities are planned or underway?*

Lead Summary 7

Title:	Investigate host pathogen interaction in ASF infection

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To gain an improved understanding of how ASFV enters, replicates and survives in and is released from infected cells.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Determine patterns of activation of immunologically relevant host genes particularly at early stages after infection.
Identify ASFV genes and genetic determinants (group of genes like multigene families) involved in host range, virulence and pathogenicity.

Solution Routes

What approaches could/should be taken to address the research question?

Dependencies

What else needs to be done before we can solve this need?

State of the Art

Existing knowledge including successes and failures

The primary cell types infected by ASFV are those belonging to the mononuclear- phagocytic system, including fixed tissue macrophages and specific lineages of reticular cells. Pathological findings in acute ASF include leukopenia B and T cell lymphopenia / thrombocytopenia lymphocyte and mononuclear cell apoptosis.
ASFV exhibit temporal regulation of gene expression. ASFV virions contain enzymatic activities that contribute to early events in, and activities critical for, viral replication in the cell cytoplasm, including RNA polymerase, nucleoside triphosphate phosphohydrolase, topoisomerase, mRNA capping, and protein kinase activity. ASFV encodes proteins predicted to mediate virus–host interaction, virulence, and mechanisms that enhance the ability of the virus to successfully replicate within the host, including homologs of cellular inhibitor of apoptosis (IAP), Bcl-2, I Kappa B (IKB) myeloid differentiation primary response antigen MyD116, lectin-like, and CD2 proteins.

Projects

What activities are planned or underway?

Lead Summary 8

Title:	Identification of ASFV antigens for antigen capture ELISA and serological tests

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
Identifying ASFV antigens to be used in serological tests and in antigen-capture ELISA

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
Detection of the same/different ASFV strains in one reaction

Solution Routes *What approaches could/should be taken to address the research question?*
Vaccine research

Dependencies *What else needs to be done before we can solve this need?*
The genome sequence of various virus isolates.

State of the Art *Existing knowledge including successes and failures*

Projects *What activities are planned or underway?*

Lead Summary 9

Title:	ASFV genome characterisation

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

There are a range of viruses' strains which differ in terms of virulence. Having whole-genome sequences is essential for identifying the host pathogen interactions and how this can be manipulated. Establishing the genomic differences of the various strains will assist in the identification of virulence mechanisms.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

The annotation and analysis of genomes in the size range of ASFV is difficult and requires specialised tools, especially with the acquisition of more genome sequences.
Automation and standardisation of viral genome sequencing for subtyping ASFV strains using NGS pipelines and automatized annotation tolls.
ASFV sequencing in ticks.
Identify key variable regions of the genome for epidemiological purposes (without need for WGS in all cases/for all outbreaks).

Solution Routes

What approaches could/should be taken to address the research question?

NGS pipelines or ASFV sequencing.
Use of bait technology for enrichment of ASFV genomes (*e.g.* with MyBait-Technology)

Dependencies

What else needs to be done before we can solve this need?

NGS laboratory with experience in large DNA genomes. Combination of different NGS technologies (large reads with MinIon and deep sequencing with standard Illumina or IonTorrent NGS).

State of the Art

Existing knowledge including successes and failures

To better understand the molecular epidemiology of the recent outbreaks, additional genome markers are under investigation. Among them are different intergenic regions. Next-generation sequencing could be aided by enrichment through targeted sequence capture technology. Using current, very robust technologies, it has been possible to develop a comprehensive database, which includes full length genome sequence of large number of isolates to replace the current less meaningful genotype-based classification: https://virology.uvic.ca/organisms/dsdna-viruses/asfarviridae/

Projects

What activities are planned or underway?

Lead Summary 10

Title:	Improving sequencing of ASF genome

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The complete sequencing of 1-3 isolates from each genotype, a series of viruses (>10) with different virulence and, a series of viruses (>5) that have replicated exclusively in domestic pigs, wild pigs and ticks.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Optimised and harmonised workflows are needed for next-generation sequencing.

Solution Routes

What approaches could/should be taken to address the research question?

Dependencies

What else needs to be done before we can solve this need?

State of the Art

Existing knowledge including successes and failures

Using current, very robust technologies, it has been possible to develop a comprehensive database, which includes full length genome sequence of large number of isolates to replace the current less meaningful genotype-based classification: https://virology.uvic.ca/organisms/dsdna-viruses/asfarviridae/

Projects

What activities are planned or underway?

Lead Summary 11

Title:	Improve direct identification of the ASF

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The improvement of direct identification of the ASF, especially direct ASFV detection via pen side testing.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Further assessment of field-applicable PCR machines or other genome detection techniques.

Solution Routes

What approaches could/should be taken to address the research question?

Easy to use point-of-care molecular diagnostics.

Dependencies

What else needs to be done before we can solve this need?

Improved DNA extraction
Direct PCR

State of the Art

Existing knowledge including successes and failures

The most commonly used techniques for virus detection and identification are haemadsorption (HA), direct immunofluorescence (DIF), and since 2000, the molecular detection of ASF virus by PCR. While HA and DIF tests are not commercially available everywhere, several qPCRs are now on the market and licensed in several countries.
qPCR is a very suitable gold standard for ASFV detection.

Projects

What activities are planned or underway?

Lead Summary 12

Title:	Identify/develop cell lines that replace primary cultures for improved virus isolation techniques

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

In order to replace laborious and homogeneous primary cultures for virus isolation, cell cultures need to be found that support ASFV replication.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Identify/develop cell lines that replace primary cultures for improved virus isolation techniques.

Solution Routes

What approaches could/should be taken to address the research question?

Systematic screening of cell lines.
Development of recombinant cell lines.

Dependencies

What else needs to be done before we can solve this need?

Discovery of the virus cellular receptors, immune markers of infection, and the pathways involved in virus replication and virus virulence.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 13

Title:	Investigating alternative sample types for ASF diagnosis

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Identify suitable sample types allowing for more environmental and less invasive diagnostic, and for easier and lower risk sample exchange.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Full validation of novel or modified direct and/or indirect tests in alternative sample types (*e.g.* blood, exudate´s tissues, oral fluids, meat juice, filter papers, swabs), including nonclinical ones (*e.g.* soil, straw, pork products, bad quality samples from wild animals).

Development and evaluation of non-invasive sampling methodologies in wild suids.

Using sampling protocols to collect samples which ease to exchange between the countries (*i.e.* no biorisk samples).

Solution Routes

What approaches could/should be taken to address the research question?

To improve reagent stability, different strategies such as lyophilisation could be explored.

Dependencies

What else needs to be done before we can solve this need?

State of the Art

Existing knowledge including successes and failures

The field of diagnostics has expanded into the search for new models of detection, including for new types of samples like faeces, FTA cards, dry blood swabs, and oral fluids, but also searching for new models as air samples and feed.

Projects

What activities are planned or underway?