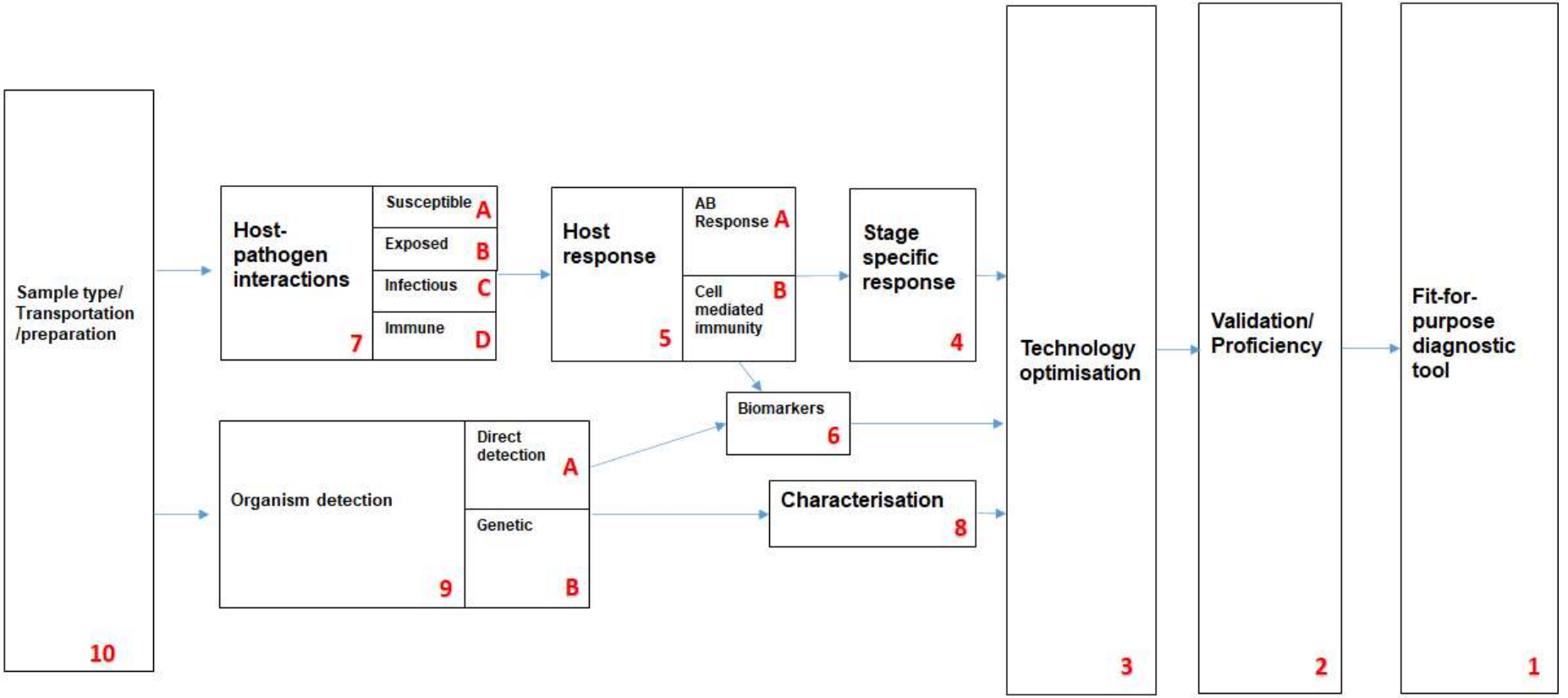
STAR-IDAZ
International Research
Consortium on Animal Health

Roadmap Lead Summaries

Disease/pathogen	Influenza					
Roadmap type	Development of Diagnostic tests					
Version: Date	V1	17/03/2023				

Diagnostic Test Development Roadmap



Lead Summary 1 - Diagnostic

Research Question

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

Development of rapid, reliable diagnostic test for early detection of influenza virus including viruses with zoonotic and/or pandemic potential and including pen-side tests

Challenge(s)

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

Subtype-specific testing capability, including antibody tests for sera
Automatic statutory reporting requirements for High Path viruses
Commercial market of diagnostic tests
Multiple antibodies to different IAV
Utility/sensitivity of in-field diagnostic solutions

Solution Routes

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

Development of tests for use in the field
Development of DIVA tests
Fast typing of AI virus using molecular tools without virus isolation, applicable for
Pets diagnostic tests may need to be developed, but lack of commercial market is disincentive for development
Tests for the identification of seroconversion resulting from IAV infection include both non-specific and multiplex assays
Assays to identify HA binding specificity for sialic acid-terminated glycans
Improve Real-Time PCR tests for subtype-specific and internal controls
In-field direct to sequencing, improved laboratory sequencing
Testing for low resource settings that don't require infrastructure

Dependencies

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

DIVA surveillance protocols acceptable to trade partner
Sample transportation and quality
Local test availability
Way to capture and share field observations and knowledge obtained during an outbreak to help prevent and control disease in subsequent events
Adequate/standardized protocols for sampling and extraction
The lower accuracy of the rapid test ,a kind of limitation, needs to be understood and accepted by the users and governments

State of the Art

Existing knowledge including successes and failures

Serologic DIVA strategies are available, but little information is available to understand how this can be used in the field (How many samples need to be taken to meet surveillance needs)

Projects *What activities are planned or underway?*

Lead Summary 2 – Validation

Research Question *What are we trying to achieve and why? What is the problem we are trying to solve?*
Validation of diagnostics tools

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
Validation of existing diagnostics against novel isolates Lack of harmonized validation protocols Development of internal controls that are standardized and readily shared Validation of portable PCR Validation of serologic DIVA tests to understand levels of sampling to achieve goal to show freedom from disease Understand how NGS can be used to supplement/augment PCR testing Validation of use for minor species (9000 avian species!!) and age of animal (e.g. piglets and sows) The chances of independent and external validation are rare and its cost may be expensive Lack of reference material

Solution Routes *What approaches could/should be taken to address the research question?*
Standardization of methods across labs – ring trials

Co-ordinated lines of reference material production Orchestrated collections of field material Improve broad detection, sensitivity and specificity of diagnostic tests Validation of portable PCR Validation of serologic DIVA tests to understand levels of sampling to achieve goal to show freedom from disease Understand how NGS can be used to supplement/augment PCR testing Public/private partnerships – letting private companies have access to samples and share technologies with companies despite restrictions in sampling handling of material from HPAI/regulated diseases

Dependencies *What else needs to be done before we can solve this need?*
Reference materials Organizatorial structures of concerted validation lines. Availability of economic resources

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

Projects *What activities are planned or underway?*

Lead Summary 3 - Technology optimisation

Research Question

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

Continued improvement of technologies

Challenge(s)

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

Decrease time and costs
Improve NextGen Sequencing for a cost-effective use in the field
Continue to optimize rRT-PCR to support routine and targeted surveillance
Pen-side application: RT-rtPCR and sequencing.
What can go wrong will go wrong in the field

Solution Routes

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

Benchmark gold standard versus new technology (PCR vs Metagenomics)
Overcoming patent issues? Capitalising on human medicine/dx to bankroll animal medicine/dx
Validation testing conducted in parallel to reduce time to release on market
Improvement of NextGen Sequencing: a) decrease the costs b) Continue to develop Minion and other rapid NGS platforms for more rapid targeted and random sequencing c) Improve sensitivity of NGS platforms d) Improve bioinformatic analysis pipelines and provide technology to be usable at state diagnostic laboratories e) Identify faster and more efficient sample preparation and testing
Optimisation of rRT-PCR: a)Continue to review and update as needed for type A and subtyping tests (use of rapid sequencing approaches) b) Continue to explore and validate environmental testing c) Integrate internal positive control to identify false negative results (possible host control) d) assemble Rt-qPCRs to match the circulating HPAIV sub- a
Local and centralized tools/staff support for data evaluation and sequence interpretation and visualization/sharing
Improvement of communication and data sharing: selected tests or diagnostics need to be used and shared by many players

Dependencies

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

Continue to harmonize protocols and adapt to new technologies
Sample collection and transportation independent of cold chain
Provide copy-based, non-infectious reference material and controls
Public/private sharing
Develop strategies to use tests (screening to characterization)
Reduce cost of rRT-PCR to increase use
Development of alternative reagents to ~~do~~ not be dependent on single manufacturer

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?	(1)A research project exploiting Nanopore technology to identify and characterise the field clinical samples quickly and accurately (’23-24)

Lead Summary 4 - Disease stage specific response

Research Question

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

Challenge(s)

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

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?

Clear definition of disease stages across species

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 5 - Host response

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

Identify a measurable host response

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

Complexity of interwoven factors
Inherent variability of maternal antibodies
Varying types of antibodies, deltaFcIgY and their roles, mucosal immunity
Need for animal models in the laboratory
Many different wild bird species as a host to understand

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

Antibody detection vs. Protection, methods for hi throughput HIs?
Other?
Utility of in vitro systems

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

Avian immunology for various species
Understanding of the immune system of migrating birds
Research facilities that can house dams, mares, hens and not just their offspring
Understanding the nature and effectors of resilient AIV immunity
Factors that contribute to failure to mount such immune responses in a species-dependent manner

State of the Art
Existing knowledge including successes and failures

Projects
What activities are planned or underway?

Lead Summary 5A - Antibody response

Research Question

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

Rapid and easy antibody response detection

Challenge(s)

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

Rapid and easy detection of neutralization antibody, cross-reactivity antibody and antigenic characterization.
Sensitvity of rapid assays
Inability to interpret antibodies in young animals – are they neutralizing? Are they maternally derived? Does their presence impact disease expression (VAERD or protection)?
Lack of true correlates of protection versus seroconversion
Understanding of the generation and decline of the humoral immunity of the infected and vaccinated birds

Solution Routes

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

ELISA and neutralizing antibody test development.
In-field rapid test development and improvement
Antibody test platforms to evaluate cell mediated immune responses.
Antibody test platforms to evaluate neutralizing antibodies.
Orchestrated development of monoclonal antibodies

Roadmap for the varying uses of antibody diagnostic approaches for veterinarians/responders based on purpose (DIVA vs. Early detection, etc)

Dependencies

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

Monoclonal antibodies availability
Reference material collection
Field sample collection

State of the Art

Existing knowledge including successes and failures

(2)Competitive ELISA based on NP protein is developed, and this can be used for the serum samples for many different avian species to see the seroconversion due to vaccination or natural infection.

Projects

What activities are planned or underway?

Lead Summary 5B - Cell-mediated immunity

Research Question

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

Diagnose the presence and valence of cell-mediated immunity against AIV

Challenge(s)

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

High laboratory specialization and technical skills of staff required
Limited understanding of variation of mechanisms between species and viral strains
Need for specific equipment to measure immune cells (I.e.: FACS, etc)
Variable genetic backgrounds of host (I.e: commerical breeds versus backyard stocks)

Solution Routes

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

Develop technically undemanding and cost-effective tools to define (parts) of CMI
Role of cell-mediated immunity (CMI) in individual animal recovery and outbreak outcomes
Tools to define immune cell populations in avian species
Test to predict cell mediated levels and correlate with vaccine protection

Dependencies

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

Basic data on the development of cellular immune effectors in various avian species
Research facilities that can hold animals for a long period of time (months) to determine if CMI is protective against heterologous challenge

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 6 – Biomarkers

Research Question

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

Biomarkers for influenza that can capture different infection phases

Challenge(s)

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

Biomarkers not known

Solution Routes

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

Determine biomarkers that can better capture infection phases, particularly for low pathogenic influenza viruses, I.e. acute phase proteins.

Dependencies

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

Validated surrogate markers

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 7 - Host-pathogen interaction

Research Question

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

Identify the most appropriate target for diagnosis based on the Host-pathogen interaction

Challenge(s)

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

Lack of knowledge on the impact of the host's infection status on the (immune) response
Virus-host interactions control species-specific pathology.
Virus-specific host susceptibility, including carrier state.

Solution Routes

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

Need to better understand the relevant mechanisms for an effective immune response, role of cross-reactivity and cell-mediated immunity.
Need to understand resistance or susceptibility factors (e.g. intracellular enzymes in different organs, endosomal pH, adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), tumor necrosis factor (TNF) etc.) towards HPAI viruses in different wild (main reservoirs) and domestic bird and mammal species
Role of microRNAs in modulating pathogenesis
Host responses for diagnosis of infections, for clues about viral pathogenesis

Dependencies

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

Challenge studies that provide foundational information
Longitudinal studies

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 7A- Susceptible

Research Question

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

Challenge(s)

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

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

Projects

What activities are planned or underway?

Lead Summary 7B – Exposed

Research Question

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

Challenge(s)

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

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

Projects

What activities are planned or underway?

Lead Summary 7C – Infectious

Research Question

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

Challenge(s)

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

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

Projects

What activities are planned or underway?

Lead Summary 7D – Immune

Research Question

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

Define duration and valence ("how protective is it?") of immunity following

- Natural infection
- Vaccination
- After multiple exposures

Challenge(s)

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

Define assays that help to evaluate the valence (protectivity) of immunity after multi exposures or multi immunizations

Solution Routes

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

Conduct longitudinal studies in naturally infected and vaccinated avian species

Determine the efficacy of natural and vaccinal immunity by virus exposure (challenge studies)
Maternal antibody interpretation, efficacy

Dependencies

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

Challenge studies
Availability of field viruses
Facilities to challenge various ages of animals
Establish standardized challenge protocols that take into account harmonized calculation of Ro.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 8 – Characterisation

Research Question

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

Characterisation of influenza virus (biological, sequences, viruses, phenotype)

Challenge(s)

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

Characterise determinants of high- vs. low-pathogenicity avian influenza also outside the HA cleavage site
Inability/ lack of ease in detecting true reassortment vs. Contamination of sample
Characterize zoonotic propensity
Limitations for in vivo testing due to animal welfare laws
Characterize antigenic properties with respect to vaccine susceptibility
Failure to maintain sample authenticity thus inability to study observed outcomes of infection
Transparency about human impacts
Lack of understanding how genomic reassortment is made exactly

Solution Routes

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

Loss of function experiments both in vitro and in vivo
Study virus transmission across interfaces
Identification of antibody epitopes important for antigenic drift in swine and poultry species
Development of models to predict epitopes based on HA sequence evolution
Promotion and adoption of a standard classification scheme (e.g., the global swine clade classification scheme and the H5 clade classification scheme) - and have this classification updated in real time (I.e.: standardized nextstrain, etc)

Dependencies

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

Virus isolates
Pathogenicity models
Interface transmission models
Genomic surveillance in multiple hosts and even different sites within host

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 9 - Organism detection

Research Question

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

Detection of influenza viruses in various species and matrices including environmental sources

Challenge(s)

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

Sensitivity of detection versus specificity versus contamination risks
Cost effectiveness, commercialization
How many/much samples are needed to determine prevalence/risk? I.e.: pools, environmental samples, versus direct from animal

Solution Routes

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

Sampling methods and transport for low resource settings
Assessment of local need to reach acceptable confirmatory diagnosis
Implementation of routine targeted testing for endemic IAVs that can be scaled up during an outbreak and/or serve as background data when a new IAV strain emerges or is a concern
Validate and verify novel solutions (field PCR, LAMP, etc) for in-field and laboratory methods
Standardize diagnostics from environmental samples
Surveillance sensitivity should be decided
Protocols to work through co-infections in order to capture all pathogens
Rapid sensitive and specific methods for field detection (subtype specific if possible?)

Dependencies

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

Clear country requirements for diagnostic (matrices and species)
Defining diagnostic needs according to region, poultry vs wild birds vs livestock vs humans etc
Understanding IAV ecology for a location/system in order to target surveillance

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 9A - Direct detection

Research Question

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

Rapid direct detection of influenza viruses and its variants

Challenge(s)

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

Cost-effective and rapid direct detection of replication-competent influenza virus
Matrix-specific effects
Overcoming embryonated eggs as the main propagation vehicle-retaining sample authenticity (re sample passage in cells/eggs)
Host antibodies that neutralize virus
Field based diagnostic for rapid early assessment with perhaps lab based confirmation/subtyping
Genetic changes associated with different systems
Low viral load in the field samples
Mixed subtypes in field samples (coinfections)

Solution Routes

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

Improvement of virus isolation and propagation by new in vitro cell culture techniques
Improvement of systems for viral replication/growth
Build cell culture collections
Modify existing cell lines to be more sensitive by expressing or repressing genes
Pen-side tests?

Dependencies

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

Availability of SPF eggs in low resource settings
Cell culture collections
Crispr/Cas technology with avian cell culture systems
Germ line manipulation tools in avian species
Sharing of resources
Early assessment linked with automatic alerts to advisors/central labs for additional tests

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 9B – Genetic

Research Question

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

Rapid, cost-effective tools for

- genetic detection
- Sequence analysis and characterization
- Sequence reporting/databases that communicate with each other (I.e., sequences from one database can be downloaded and analyzed in another without loss of integrity)

Challenge(s)

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

Systematic approaches for analysing and reporting viral genome sequences
Issues around samples most relevant for infection in different species with swabs alone not necessarily enabling detection
Approaches that are specific ie need to know what you have before you can test

Solution Routes

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

Effective deployment of 3rd -gen sequencing technologies for both wet- and dry-lab protocols, allowing point-of-incidence analysis
Rapid characterization of variants circulating in the field via high-throughput point-of-care technology
Improve sensitivity and purification methods for whole-genome sequencing
Evaluation of whole genome sequencing platforms
Develop highly multiplexed PCR assays
Improved and easily employed metagenomic pipelines
Commercialization or Increased availability of novel technology like viability PCR tests

Dependencies

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

Training of more bioinformaticists to mine and interpret the data
Understand the relationship between genetic detection and transmission IRL
Improved reference databases/faster assignment of genetic clades as they appear
Turnkey solutions to allow use in more veterinary labs
Reduce complexity of testing procedure to make it more accessible

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

(3)A Research project for development of multiplex real-time RT-PCR for subtypes H5/H7/H9 and multiplex end-point RT-PCR of HA subtyping (’22-23)

Lead Summary 10 - Sample type/transportation/preparation

Research Question

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

Improvement of viral sampling, transportation and isolation of nucleic acids and viruses

Challenge(s)

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

Sampling sparing methods
Improvement of safe transport of virus
Pools and matrix of choice for environmental sampling, Virus extraction from environmental matrices (e.g. water, mud, aquatic microfauna, etc.)
Poultry environs that also include varying types of chemicals
Limitations defined by CITES (species protection and conservation acts)
Limitations defined by the NAGOYA protocol (WHO exemptions etc.)
Preserve the quality and viability of biological samples during transportation over long distances and temperature extremes

Solution Routes

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

In-field extraction and diagnostics (PoC/Penside) to minimize transport
Development of transport media that stabilise the virus without refrigeration
Non-invasive samples from wild birds
Non-invasive samples from swine
Environmental sampling methods including

- Air sampling and analysis protocols
- Waste water
- Poultry house sampling, where are the best samples

Dependencies

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

Standardise environmental sampling procedures
Easy legal frameworks for transport
Manageable CITES and NAGOYA rules for avian samples
Data management for sampling/testing coordination
Human resources

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

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