STAR IDAZ | International Research Consortium on Animal Health

Roadmap Lead Summaries

Disease/pathogen	Coronavirus					
Roadmap type	Development of Diagnostic tests					
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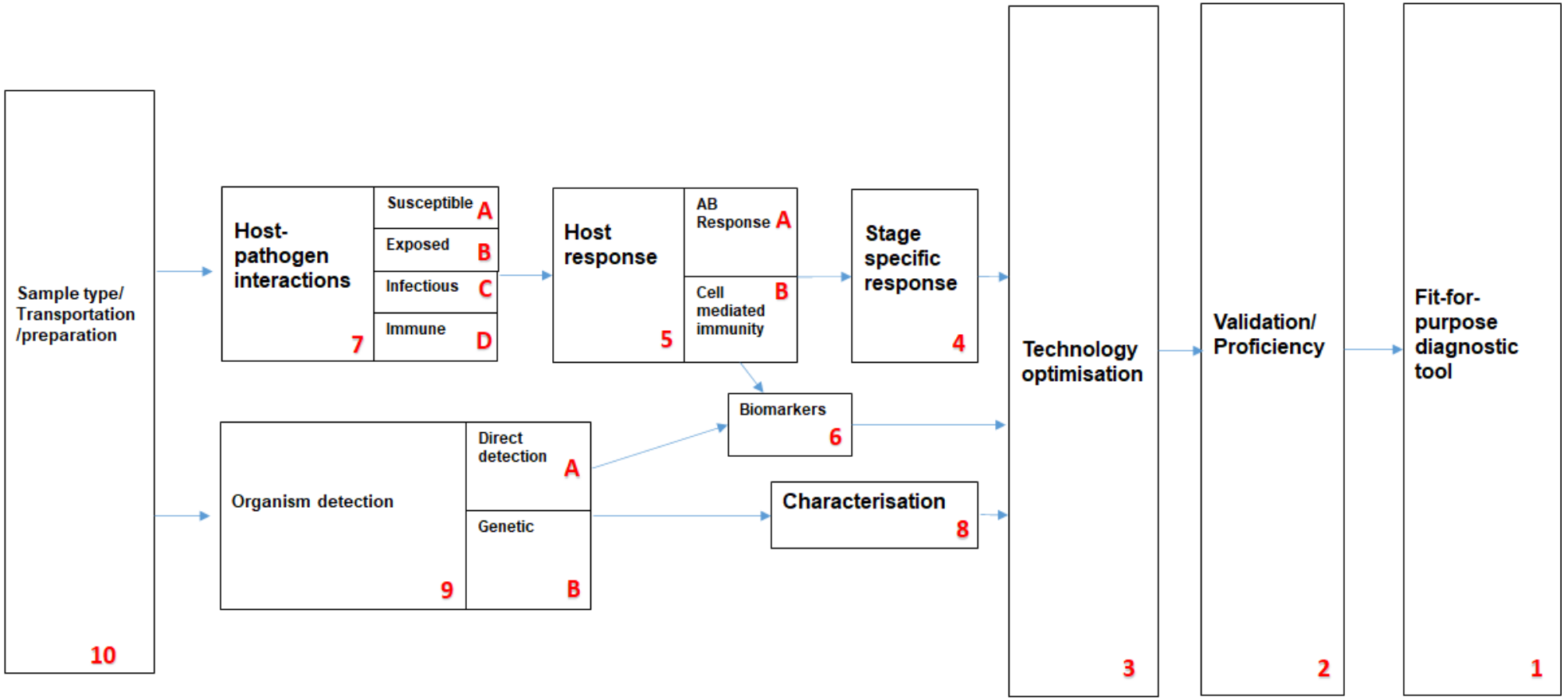
	v.1.1	September 2024				

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 detection of CoVs and CoV infections in animals including strains 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)?

Detection and typing of different coronaviruses with different genome constellation
Commercial market of diagnostic tests
Detection of immune responses for different host species
Easy sampling for wildlife
Accessible costs
Utility/sensitivity of in-field diagnostic solution
Understand which coronaviruses are relevant and with zoonotic potential
Develop different type of diagnostic depending on the end user (e.g. government or farmers) and the aim (e.g. Clinical Diagnostics vs. Surveillance- normal/healthy vs ill vs. recovered)
Pan coronavirus essays
Select the best matrix for non-invasive test to avoid licencing issues (e.g. aeresol or faeces)

Solution Routes

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

Development of Point of Care Test (POCT) panCoV tests for use in multiple host species (companion animals, agricultural/livestock and wildlife). Find the Agent (FTA) card to transport genome without cold chain, for coronaviruses this needs validation/accreditation
Lab-in-a suitcase PCR/nanopore sequencing
Development of bioinformatics pipelines from raw reads to report for short and long reads coronaviruses sequence analysis
Improved laboratory standard operation procedures (SOPs) sequencing CoV genomes
Testing for low resource settings that don't require infrastructure
Metagenomic approach to virome/pathobiome could be utilised to characterise pathogens when the traditional diagnosis has not been reached
Co-design of tools with farmers/final users – consider end users
Rapid and cheap testing to distinguish lineages
Detection of antibodies in postmortem samples (wildlife)
POCTs with high specificity and sensitivity, possibly a multispecies lateral flow panCoV test for surveillance, followed up by lineage specific testing, either as advanced pen-side or laboratory tests.

Dependencies

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

Policies that allow faster approval of new technologies across national borders Flexible regulations/standards based on updated scientific evidence Biobanking for coronaviruses Capacity for quick scale up of products in emergencies Overcome political and misuse challenges
State of the Art

Existing knowledge including successes and failures
A detailed list of diagnostic test available in animals can be found here: List of Animal Health Diagnostics - Diagnostics For Animals
Projects *What activities are planned or underway?*
Preparing for emerging pathogens (PREP4EP)- Netherland

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 diagnostic 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
Numerous potential host species/sample types, need host/sample agnostic approach or broad as possible
Development on internal controls that are standardized and readily shared
Validation of portable PCR tools
Understand how NGS can be used to supplement/augment PCR testing
Lack of reference materials/regulatory reference material distribution challenges
Sample integrity can be an issue with field samples
Validation for different species, including companion animals, and different matrix
Consideration of in-field conditions for POCT and ease of use

Solution Routes

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

Organization of ring trials in Corona PCR and NGS
Co-ordinated lines of reference material production
Improve broad detection, sensitivity, specificity and typing
Validation of portable PCR
Public/private partnership
Validation of portable PCR for PanCoV (pan-SarbeCoV, pan-MerbeCoV) testing
Available reagents (for non-human hosts?)
Establishment of an international collaborative biobanking (virtual biobanking) system

Dependencies

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

Organization of structure of concerted validation lines

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
Knowledge on antibody-based/serological cross-reactivity between CoVs
Improve Pan CoVs tests
Improve NGS technologies and bioinformatics analysis
Pen-side applications for rapid diagnostics, particularly in low-resource settings
Interpretation of technologies/assay results in different host species

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

RNA and serological assay combined
Benchmark gold standard versus new tech
Public-private partnership
Improve NGS for easier usage in the field and affordable costs
Facilitate bio-informatic data sharing; facilitate typing

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

Continue to harmonize protocols and adapt new technologies
Sample collection and transportation independent of cold chain
Provide reference materials and clinical material for sharing – Nagoya protocol problems with sharing local lab capacity
Reduce costs of PCR based assays to increase use
Public/private partnership

State of the Art
Existing knowledge including successes and failures

Projects
What activities are planned or underway?

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?*
Understanding the asymptomatic infection role in infection transmission

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
Relation between nucleic acid/serologically +ve patients and infectiveness Disease stage specific response is highly dependent on specific CoVs and the host species Discover how enteric CoVs differ to respiratory CoVs Diagnostics of asymptomatic cases

Solution Routes *What approaches could/should be taken to address the research question?*
Approach it in a case-by-case manner
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 and generation of positive control reagents

Challenge(s)

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

Understand how well SPF animals mimic natural infections (e.g. Consideration for inbred vs outbred lab animals)
Characterisation of natural history/growth kinetics of CoVs in appropriate animal models – comparison of acute vs established or historic infections
Detection of current and previous infections
How to select the best hosts to focus on. Is the host that favours coronavirus recombination the best one?

Solution Routes

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

Longitudinal studies on animals naturally exposed to different coronaviruses
Development of suitable detection platforms for different host responses

Dependencies

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

Development of *in vitro* models (e.g. organoid) to propagate infectious material for *in vivo* animal studies
Animal models and growth kinetics in each specific animal model species
Detection platform in either a species-dependent or -independent 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 detection (POC or LFT) to mitigate dependencies on virus neutralisation tests that take longer to perform

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

What is the cross-reactivity amongst different CoV species – can this be supplemented with molecular testing?
Potentially multiple CoV species circulating in animal populations
Maternal immunity in herds/individuals
Species-independent detection platforms
Collection of antibody samples from postmortem tissues or other challenging matrices
Lack of control reagents in diverse or wildlife species (i.e. secondary)
Requirement for expertise and facilities for BSL3

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

Purification of antigens from different CoVs
Study decline of humoral immunity of infected animals in different species
Development of novel platforms for species-independent detection
Development of surrogate testing to be performed at CL2/BSL2 in the absence of BSL3 – consideration that pseudotypes/VLPs may not fully recapitulate whole virus conditions
Monoclonal antibody competition ELISA for cross-(host) species antibody detection

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

Monoclonal Abs availability
Rapid sharing of reagents

State of the Art
Existing knowledge including successes and failures

Multispecies N protein ELISA is available
Several S specific ELISA are available too
Multiplex surrogate virus neutralization test (sVNT)

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?

Can T-cell immunity be used as a diagnostic tool for detection and surveillance for animals?

Challenge(s)

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

Tests are time consuming and expensive
Unclear understanding of cell-mediated immunity in some species the basic immunology is not well characterised
High species-dependence

Solution Routes

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

Development of relatively rapid T-cell assays
In highly important cases, T-cell can be used in high-value samples

Dependencies

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

Availability of new and rapid platform
Specific knowledge in certain animal species

State of the Art

Existing knowledge including successes and failures

T-cells, especially memory T-cells, play a crucial role in long-term immunity against SARS-Cov-2.

Projects

What activities are planned or underway?

Duke-NUS medical school currently use T cell assay to detect past infections – long lasting immunity and specific diagnosis

Lead Summary 6 – Biomarkers

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

Identify biomarkers for coronaviruses
(see sections 5 -biomarkers for host response- and 7 -host-pathogen interaction)

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

Are immunological antigens common amongst different CoVs?
Difficult to identify specific biomarkers for diagnostics e.g. for FIP

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

Study conserved regions of N protein for pan coronavirus serological detection

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 7 - Host-pathogen interaction

Research Question

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

Host pathogen interaction for developing a realistic diagnostic tool to address susceptible (7A) and Immune (7D)

Challenge(s)

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

Susceptibility and pathogenesis of sarbecoviruses/merbecoviruses and embecoviruses etc is not completely understood.
Signs due to immunopathology rather than virus specific pathology
Distinguishing previous cross-reactive antibodies on ELISAs (e.g. Canine CoV inducing Ab which can be detected on Feline CoV test makes difficult to distinguish between exposure to different Coronavirus)
Ct values on molecular tests do not correlate to infectious status
Possible combination of assays including molecular and antibody tests to determine true status

Solution Routes

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

Establishment of both in vitro and in vivo testing platforms, focusing on organoid and animal models
Use of sgRNA (or replication intermediate) detection to supplement serological testing
Use of virus isolation to demonstrate infectious virus is within the sample as can't rely on qRT-PCR ct values (needs BSL3)

Dependencies

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

Identification of entry receptors for specific CoV in different animal species
Suitable testing platforms
Organoids and tissue engineering to study coronavirus interactions in relevant hosts

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?

Can we distinguish between susceptible and exposed?

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?

Can we differentiate between susceptible and exposed?

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?

How do we identify those shedding live virus with the ability to transmit to others

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?

Is there protective immunity?

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

Define protective immunity
Duration and strength of immunity vary between coronavirus type, individual factors and infection severity

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 8 – Characterisation

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

Genetic/biological characterisation of major coronaviruses impacting animal health and/or having zoonotic concerns

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

Characterize determinants of high vs low virulence
Characterization of and host range host/issue tropism
Characterization of the antigenic properties
General genetic similarity between many coronavirus lineages, therefore sequencing is needed for species identification
What features characterise new variants of concern?
Lack of standardized tools for basic biological characterization
Lack of adequate cell lines for isolation and characterization
Characterisation of major drivers for virus jumping into new species of hosts
Understanding the effect (if any) of propagating viruses in cell lines / organoids on the phenotype of the virus

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

Broad sharing of quick reverse genetics strategies-such as Insertional Somatic Mutagenesis (ISA)- to rescue synthetic viruses
Loss of function experiments by reverse genetics strategies
Study virus transmission across human/animal interface and animal/animal interface
Development of models to predict epitopes based on S sequence evolution
Improve ex vivo, in vitro and organoids characterization of new viral isolates

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

Virus isolates
Standardised animal models to characterise virus
Interface transmission models
Genomic surveillance in multiple hosts and even different sites within the same host

State of the Art
Existing knowledge including successes and failures

Projects
What activities are planned or underway?

ConVErgence (ICRAD/EU) aims to address knowledge gaps regarding the emergence of novel CoVs in swine trough spillover from humans and bats, and to provide a genetic and biological characterization of emerging CoVs, including the possible host range in bats and the zoonotic potential.

Lead Summary 9 - Organism detection

Research Question

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

Coronavirus detection in different species and matrix

Challenge(s)

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

Cost-effectiveness of sampling strategy
Interpretation of detection or assay results in different species/matrices, i.e., setting the specificity and sensitivity in different species context
Field tests associated with early reporting systems
Sampling from wildlife (e.g. bats)
Organism detection requires active infection or presence of virus nucleic acid, not indicative of exposure

Solution Routes

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

Protocols to work through co-infections in order to capture all pathogens (virome/metagenomics)
NGS for in-field application and affordable
Rapid sensitive and specific methods for field detection (pen-side or LFT)

Dependencies

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

Prediction models
Defining diagnostics needs according to region, species etc
Understanding specific CoV ecology in order to implement surveillance

State of the Art

Existing knowledge including successes and failures

Organism detection is particularly important in herd diagnostics when antibody response might be unclear, e.g. other infections and/or young age for maternal immunity

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 detection of CoVs in different species and matrix

Challenge(s) *What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
Matrix specific effects Multispecies detection Cell-culture isolation of many CoVs is challenging Low viral load in field samples (lack of understanding of the disease pathogenesis = ? timing of sampling) Different genome constellations Mixed strains Unknown receptor specificity

Solution Routes *What approaches could/should be taken to address the research question?*
Improvement of VI by new systems Improvement of systems for viral replication/growth
Build broad range cell culture collections and organoids Modifying current available cells lines for being more sensitive to the different CoVs including dampened innate immunity and sensitive reporting system

Dependencies *What else needs to be done before we can solve this need?*
Basic knowledge on receptor usage Sharing of resources Training in genome manipulation technologies (CRISPR) Cell culture collections

State of the Art *Existing knowledge including successes and failures*
Direct methods are available (e.g. virus isolation, electron microscopy and virus neutralization) but, being time consuming and laborious, are not envisaged for early detection, nor for surveillance.

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?

Affordable tools to be utilised in the field for:
-genetic detection
-genetic characterization
-identification of virulence factors
-identification of homologous or heterologous genes

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

Genetic similarities between many coronavirus lineages (therefore sequencing is needed for species identification)
Field adaptation
Different species
Different matrix
Bioinformatics support/characterization/interpretation of virulence markers or genetic signatures
Consideration that during diagnostics, we may also hit upon discovery
Biosafety issues restrict viral isolation
Internal quality controls (e.g. arbitrary ct values) may bias towards detection of higher viral infectious loads and some may be missed
Consideration that successful viral isolation may introduce lab adaptations that infer rapid changes and may not reflect wild type virus

Solution Routes

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

Protocols for different matrix (milk, mucous membrane swabs, faeces…)
Develop multiplex PCR
Purification methods for sequencing
On site-trials and validation
User friendly tools and reporting systems
Bait and capture, enrichment approaches for high ct/low copy samples to generate sequences where possible
Development of multiple systems to give a preliminary assessment of biological characterisation – genetic sequence and viral kinetics to be shared widely
Implementation of appropriate biosafety measures and risk assessments to ensure safe working (and acceptability from external stakeholders e.g. general public)
Development of standardised processes for the above.

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

Effective training for performing and interpretation of data
Training in bioinformatics
Standardised protocols for different settings
Regulation for reporting – implementation of databases
Improve timely- communication with risk assessors/ policy makers

State of the Art
Existing knowledge including successes and failures

Projects

What activities are planned or underway?

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?

Standardise sampling for ease and safe transport

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

Technical and regulatory issues
Safe handling for operators
Preserve samples from variability of temperature in field condition transportation
Non-invasive sampling methods for wildlife (including restrictions on authority to sample wildlife for discovery)
Accessible matrix for livestock (e.g. bulk milk, faeces, oral fluids…)
Limitation from regulations to samples movements of high pathogenic virus (Nagoya protocol) vs need for rapid access to outbreak strains to develop diagnostics
Timeline for policy development/negotiation of sample sharing related to implementation of Nagoya protocol at country level

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

Find the Agent (FTA) card to transport genome without need for cold chain
Drone technology to speed up delivery and collection from the field to hubs for processing of samples
Standardised protocols for sampling on different species and matrix
Virtual biobanking, at least on a regional level (sera and clinical samples) with an overarching policy to allow sharing
Validate specificity of tests using banked samples
Sampling methods and transport for low resource settings
Generate synthetic virus / products (GM) from published sequence when wild types can't be shared
Utilization of postmortem tissues for antibody surveillance (i.e. meat or lymph node exudates). Especially useful in wildlife or post-harvest sample
Develop guidelines for sample sharing
Influencing policy: working with government to address export control restrictions/Nagoya protocol

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

User friendly strategy for sampling in different settings
Global standards from policy makers

State of the Art
Existing knowledge including successes and failures

Proof of concept for lymph node exudates for antibody-based SARS surveillance in US White tailed deer (Poonsuk 2023)
Existing global standards and recommendations for Coronavirus:
WOAH Terrestrial Manual CHAPTER 1.1.2. COLLECTION, SUBMISSION AND STORAGE OF DIAGNOSTIC SPECIMENS
https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.02_COLLECTION_DIAG_SPECIMENS.pdf

WOAH Terrestrial manual CHAPTER 1.1. 3 . TRANSPORT OF BIOLOGICAL MATERIALS:
https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.03_TRANSPORT.pdf
WOAH Terrestrial Manual CHAPTER 3 . 5.2 . MIDDLE EAST RESPIRATORY SYNDROME (INFECTION OF DROMEDARY CAMELS WITH MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS):
https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.05.02_MERS-CoV.pdf
WOAH Considerations for sampling, testing, and reporting of SARS-CoV-2 in animals:
https://www.woah.org/fileadmin/Home/MM/A_Sampling_Testing_and_Reporting_of_SARS-CoV-2_in_animals_3_July_2020.pdf

Projects

What activities are planned or underway?

NeResDia project: examining the stability of viral RNA from human respiratory viruses (including SARS-CoV-2) under different storage conditions – Dr Melina Hart, Austria
ISARIC project to standardise the collection of clinical samples for human SARS-CoV-2 (Liverpool) - this could be expanded upon for sampling animals