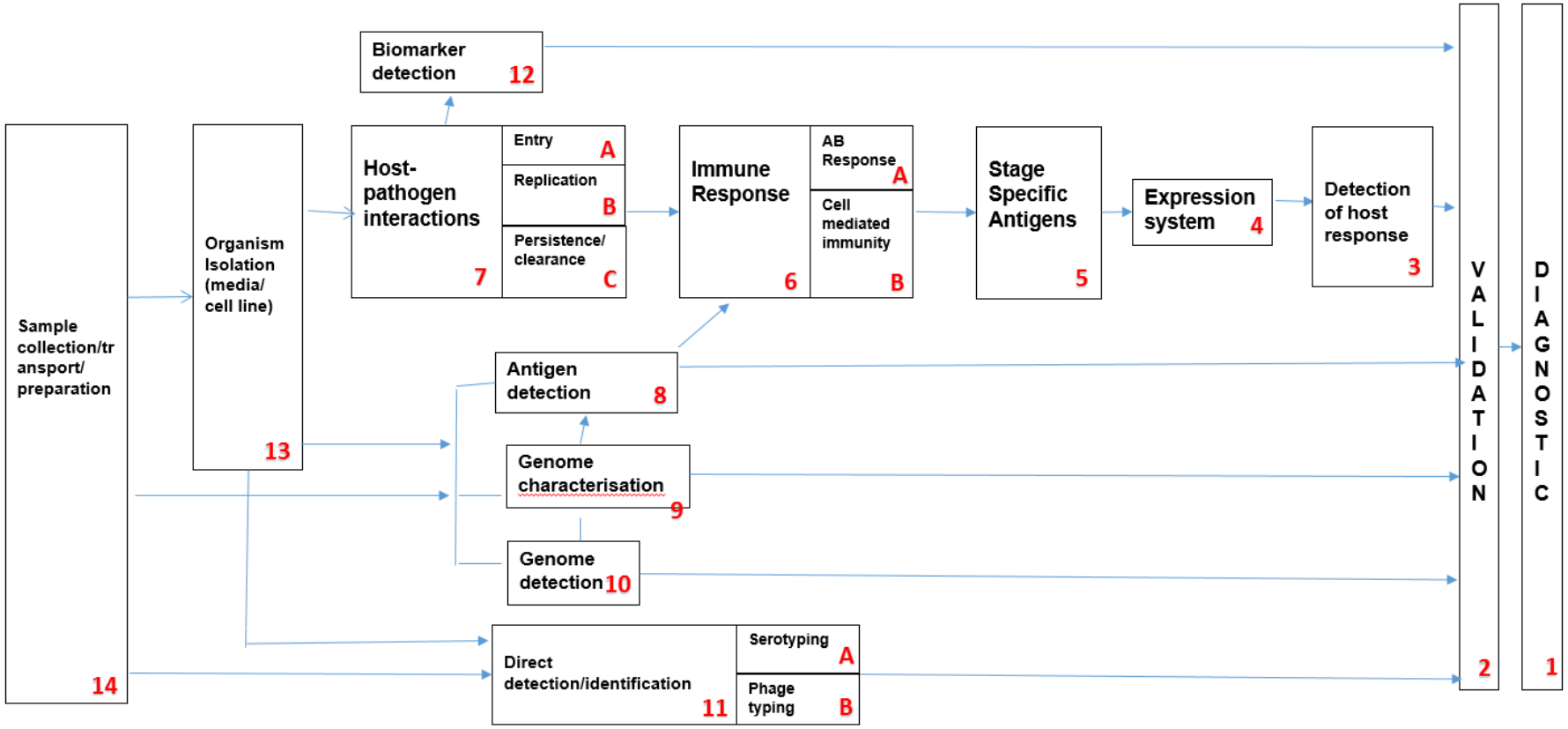
STAR-IDAZ
International Research
Consortium on Animal Health

Roadmap Lead Summaries

Disease/pathogen						
Roadmap type	Diagnostic Test					
Version: Date	V1	12/6/2019				
	V2	24/5/2023				
	V3	18/1/2024				

Diagnostic Test Development Roadmap



Lead Summary [1]

Title:	Diagnostic test development

Research Question

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

A sensitive, specific, rapid, easy test to use, pen side test for livestock and wildlife – for direct detection of infected animals (including early stage or latent infection) or the presence of infection in animal products (meat and milk) or animal excreta.
Enable Differentiation between Infected and Vaccinated Animals (DIVA)
Why is a diagnostic test important and where is it needed? How can different diagnostics be developed and optimized for different purposes:

- Detection in live animals (wildlife/farmed – surveillance)
- Single shot test, same day
- Post-mortem surveillance
- Ante-mortem test in LMIC countries

Challenge(s)

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

Cost of test
User friendliness
Availability of reagents
Evaluation, validation, and accreditation.
Increased sensitivity implies that apparent prevalence underestimates true prevalence substantially.
There are ISO-accredited lab Reference Methods (culture), no gold standard against which to compare; culture has well-documented limitations.
No equivalent to the human TB CRS (composite reference standard).
Misinformation about what bTB test results mean.
Optimal sample type?
Turnaround times.
Throughput.
Challenge to develop a testing algorithm.

Solution Routes

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

Development of tests based on host responses that are more sensitive and specific than the tuberculin intradermal skin test for the detection of infection in live animals.
Development of tests for the detection of the organism or its metabolic (biomarker) products.
Explore advanced diagnostic technologies, such as next-generation sequencing, proteomics, and metabolomics, to identify new biomarkers and improve the sensitivity and specificity of bTB diagnostic tests.

Invest in research efforts to discover and validate novel biomarkers specific to bTB infection, disease progression, and treatment response, leveraging omics technologies and comprehensive molecular profiling.
Consider CRISPR-based diagnostics.
Develop molecular methods, including genotyping and whole-genome sequencing, to accurately differentiate and classify various bTB strains, including zoonotic and pandemic potential strains.
Invest in leveraging technologies such as isothermal amplification, lateral flow assays, and miniaturized lab-on-a-chip devices for on-site bTB testing.

Dependencies

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

Test validation in relation to sensitivity and specificity, Positive (PPV) and Negative Predictive Value (NPV) , yield and cost-benefit analyses.

Agree optimal sample type(s), protocols and timings.

State of the Art

Existing knowledge including successes and failures

Current tuberculin skin test.
Gamma interferon.
Culture based detection.
PCR based detection.
AB based systems.
Cepheid GeneXpert and Ultra used in human TB reference labs.
Synthetic, defined purified protein derivative PPDs being trialled.

Projects

What activities are planned or underway?

Lead Summary [2]

Title:	Test validation

Research Question

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

To establish fit for purpose performance specifications and enable assessment of proficiency

Challenge(s)

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

No gold standard or Composite Reference Standard against which to compare test performance.
Acceptable standard for whether or not an animal is infected.
Establishment of proficiency panels.
Better understanding of risk categories. Defined criteria – requirement for specificity and sensitivity, PPV, NPV etc.
Well-documented interaction between prevalence and test performance; variation of a test's sensitivity and specificity with disease prevalence

Solution Routes

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

Data on reproducibility/repeatability of immunological or molecular tests.
Availability of well-characterised (anonymous) biobanks of biological materials i.e., serum, blood, tissue etc., including experimentally infected animals, controls, and field cases.
Validation requirements from WOAH etc.
Detection trials in animals experimentally infected with *M. bovis*.
Detection trials in animals experimentally infected with related Mycobacteria.
Inter-laboratory proficiency ring trials.
Field trials in known reactor animals to confirm infection status.
Large scale field trials.
Conduct extensive field validation studies to evaluate the performance, feasibility, and usability of new diagnostic tests in diverse bTB epidemiological settings, considering factors such as sensitivity, specificity, cost-effectiveness, and ease of implementation.
Develop robust data management systems that enable efficient collection, integration, and analysis of bTB diagnostic data.
Utilize advanced analytics, artificial intelligence, and machine learning techniques to extract meaningful insights and support evidence-based decision-making.
Developing and adhering to standardized protocols and guidelines to promote consistency and reliability in bTB diagnostics.
Consider latent class analyses of test performance.

Follow STARD and QUADAS-2 guidelines for reporting and designing diagnostic accuracy studies.

State of the Art

Existing knowledge including successes and failures

Dependencies

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

The development on novel tests of host immune responses and direct/indirect pathogen detection in infection.
The development of novel tests that are dependent on biomarkers other than those related to the immune response of the host

Projects

What activities are planned or underway?

Lead Summary [3]

Title:	Tools for detection of host response/organism at the individual level

Research Question

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

A reliable test to detect infection at various stages.
Options include biomarker detection of the host response or the organism.
To develop biosignatures (combination of biomarkers) that could inform on the latent/carrier status of an animal and evaluate the risk of Mb shedding and transmission .
Development of tools to identify animals at risk to transmit for targeted elimination to end massive herd culling

Challenge(s)

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

Define the stages of infection, i.e., is it like human TB hypothesis?
Current detection systems for cell-mediated immune responses need improvement.
Trade-off between test antigen specificity and sensitivity.
Detecting shedders.
Differentiating infectious from infected animals. Current tests cannot distinguish exposure from infection; precautionary principle means all immunoreactive cases removed.
Test should differentiate infected from vaccinated.

A deeper understanding of the host-pathogen interaction and the development of sensitive and specific biomarkers.
Understanding the dynamic nature of host immune responses during different stages of bTB infection is crucial.
Combination of different biomarkers (mix omics) to define biosignatures of infection
We need to understand the different clinical states of TB in cattle akin to what is now establish for human TB
We need to develop easy and affordable tools to identify biosignatures
We need to identify a biosignature in circulating blood (the easiest and less invasive procedures for sampling large numbers of animals)

Solution Routes

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

Improved skin test with characterised (molecular defined) antigens.
Establishment of standard banks of tissue, cells and serum from animals with known infection status.
Serological test.
Interferon γ test with characterised antigens.
Other tests of Cell-Mediated Immune responses.

The identification of biomarkers that can differentiate between early-stage, latent, and active infections.
Multiplexed analyte/antigen testing, e.g., Luminex.
Machine learning to scan for biomarkers, e.g., infrared spectroscopy in milk and blood.
We need to embrace the One Health approach and work together with human TB specialists
Zoonotic (human) cases tend to be detected as MTBC. No structured surveillance for at risk groups; tend to present to health services with symptoms.

Dependencies

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

Known, preferably stage-specific antigens to which the host responds.

We need to establish robust pipelines with all stakeholders- including bTB surveillance programs- to ensure rigorous and longitudinal follow-up of animals in the field

State of the Art

Existing knowledge including successes and failures

Human biomarker panels. One Health Approach

Projects

What activities are planned or underway?

Lead Summary [4]

Title:	

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 [5]

Title:	Identification of Stage-specific markers

Research Question

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

To identify antigens which the host is responding to at different stages of infection. Individual antigens may only initiate responses at different stages of infection. PPD is the basis of most current tests, but it hasn't been standardised.

Challenge(s)

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

Characterisation of PPD (Purified Protein Derivative)- proteomics, glycosylation, acylation patterns.
Differentiation between exposure and infection.
Identify productive infection, transmissibility?
Differentiation between latent and active infections.
Large-scale sample testing, including animals at different stages of infection, to establish the reliability and accuracy of the identified biomarkers.
The immune response to bTB infection can vary among individuals, which can make it challenging to identify universal stage-specific antigen markers.
Ensuring high sensitivity and specificity of stage-specific antigen markers to minimize false-positive and false-negative results.
Factor in, or control for, host genetic background, i.e., susceptibility.

Solution Routes

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

Molecular fractionation and characterisation of PPD, proteins, glycolipids etc.
Preparation (synthesis) of molecularly defined antigens.
Recombinant antigens may have a role but challenging to standardise.
Development of synthetic reagents/antigens.
Longitudinal studies of host serological and CMI responses during the course of infection.

Dependencies

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

The identity of the antigens being expressed by the organism in macrophages at different stages of infection in "immune" and "resistant" hosts (in vitro or in vivo experiments)

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary [6a]

Title:	Ab Response during infection

Research Question

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

To characterise the humoral immune response during infection – can the humoral response be reliably used to identify infection status?

Challenge(s)

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

Establishing the earliest points that specific humoral responses can be detected.
Trade-off between antigen sensitivity and specificity.
The interaction between the humoral response and earlier tuberculin skin testing (anamnestic response).
Immunoreactivity indexes exposure; cannot currently distinguish from true, productive infection.
Distinguishing immune responses induced by vaccination from those triggered by natural infection (DIVA).
Identifying specific antigens or epitopes that are recognized by antibodies during bTB infection.

Solution Routes

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

Investigating the timing, magnitude, and persistence of antibody production, as well as the correlation between antibody levels and disease progression or treatment response.
Longitudinal studies monitoring the antibody response (including antibody isotype and subclasses) during experimental infection.
Overcoming challenges associated with cross-reactivity, variations in antibody responses among individuals, and differences in test performance across diverse populations.
Control for host genetic background.

Dependencies

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

Improved understanding of host-pathogen interactions.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary [6b]

Title:	Characterisation of the CMI responses during infection

Research Question

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

To characterise the cell-mediated immune response during infection to identify which cytokines and other biomarkers (transcriptomics, metabolomics…) responses could be used to detect and monitor infection.

Challenge(s)

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

Investigating the specific roles and functional diversity of different T cell subsets, such as CD4+ T cells, CD8+ T cells, and gamma-delta T cells, during bTB infection.
Understand how innate immune cells (particularly macrophages and neutrophils subsets) shape the protective vs deleterious immune response.
Challenge to control for host genetics, pathogen genetics, dose, route, concurrent infection(s) and other environmental risks.
Exploring the mechanisms involved in pathogen killing, granuloma formation, and control of bacterial dissemination.
Understanding the mechanisms by which *M. bovis* evades or suppresses the host CMI response.

Solution Routes

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

Characterizing the cytokine profiles produced by T cells and other immune cells during infection.
Identifying the key cytokines involved in the immune response, their timing and levels of production, and their correlation with disease outcome and progression.
Investigating the effector mechanisms employed by cytotoxic T lymphocytes (CTLs) and other immune cells in combating bTB infection.
Studying the development and maintenance of memory T cell responses following bTB.
Exploring the longevity, functional properties, and protective potential of memory T cells in providing long-term immunity and protection against reinfection.
Comparative studies across different host species, such as cattle and other animal models, can provide insights into conserved and species-specific aspects of the CMI response to bTB.
Control for host genetic background; be aware of spectrum of responses.
Investigating the interactions between all the above T cell subsets and innate immune cells (particularly macrophages and

neutrophils subsets) during the different stages of the immune response, in susceptible versus resistant animals
Looking into the antigenic variation, immune evasion strategies, and mechanisms of immune tolerance employed by the bacterium will aid in designing effective interventions.

Dependencies

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

Improved understanding of host-pathogen interactions.
Availability of suitable immunological reagents at a price that would make test suitable for widespread use.
Set up cohorts of animals for longitudinal follow-up of animals in the field

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary [6]

Title:	Characterisation of the immune response during infection

Research Question

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

Establish the host immune response, including what it is responding to and when.

Challenge(s)

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

Investigating the kinetics of immune cell activation, cytokine production, antibody generation, and the transition between innate and adaptive immune responses.
Cross-reactivity with other environmental mycobacteria and related organisms.
Immunoreactivity indexes exposure and includes productive infection.
Trade-off between antigen sensitivity and specificity.
Heritable variation in bTB risk and outcomes of exposure.

Solution Routes

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

Fractionation and characterisation of PPD.
Synthesis of molecularly defined PPDs.
Investigating the migration and tissue localization patterns of immune cells during infection.
Figure out how immune cells traffic to and accumulate at the site of infection, as well as their distribution within tissues; can provide insights into immune surveillance and effector mechanisms.
Elucidating the development, maintenance, and durability of immune memory following infection.

Dependencies

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

An improved understanding of the host-pathogen interaction.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary [7a]

Title:	HPI : role of innate cells (mostly macrophages and neutrophils) in early clearance of M. bovis.

Research Question

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

As indicated by epidemiological studies of human TB, there must exist animals that are exposed to M bovis in herds but do resist/clear infection without signs of exposition (TST neg) . We need to understand the innate immune system of these individuals

Challenge(s)

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

We need to define the roles of different cells of the immune system in cattle (alveolar macrophages, neutrophils and others) in uptake and clearance of the pathogen)

Solution Routes

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

We need to embrace the One Health approach and work together with human TB specialists

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]

Title:	

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]

Title:	Persistence/clearance : identification of signatures of clinical status at the individual level

Research Question

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

The detection of animals that have latent infection.
To develop biosignatures (combination of biomarkers) that could inform on the latent/carrier status of an animal and evaluate the risk of Mb shedding and transmission .
Development of tools to identify animals at risk to transmit for targeted elimination to end massive herd culling

Challenge(s)

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

Persistence implies existence of long-term, largely undetected infection; may be risk if productive infection.
Current tests lack sensitivity to index exposure and infection.
Is latency an epidemiologically important feature of bTB? How would latency manifest?
Does *M. bovis* persist in the environment?

To understand the different clinical states of TB in cattle akin to what is now establish for human TB
To develop easy and affordable tools to identify biosignatures

To identify a biosignature in circulating blood (the easiest and less invasive procedures for sampling large numbers of animals)

Solution Routes

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

We need to embrace the One Health approach and work together with human TB specialists

Dependencies

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

Longitudinal in vitro, in vivo, or animal studies.
Animal-level molecular or genome epidemiology may find signals of persistence i.e., long-term infection in repeatedly negative cases, such as lesioned at routine slaughter.
To establish robust pipelines with all stakeholders-including bTB surveillance programs- to ensure rigorous and longitudinal follow-up of animals in the field

State of the Art

Existing knowledge including successes and failures

Projects *What activities are planned or underway?*

Lead Summary [7]

Title:	Characterisation of the host pathogen interaction

Research Question

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

To establish how the host is responding and what it is responding to at different stages of infection.

Challenge(s)

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

Indistinguishable exposure and infection: current tests generate immunoreactivity but do not distinguish exposure from infection; consequently, a proportion of reacting cattle may have cleared infection. This requires empirical study.

Interaction with macrophages: *M. bovis* infects macrophages which are an important contributor to the immune response so establishing how the bacterium interacts with macrophages is central to identifying the protective mechanisms and how the bacteria evades them.

To enhance the killing of infected immune cells.

Variability in animal resistance: compelling evidence that animals vary in their resistance to *M. bovis* infection; there is heritable and exploitable variation in how animals respond to *M. bovis* exposure.

Genotypic variations of *M. bovis*: *M. bovis* populations can be genotyped into various lineages and clones globally. These genotypes may have epidemiologically relevant phenotypes at the clone level. This remains to be demonstrated empirically.

Solution Routes

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

Relatively high risk and low risk cattle sires can be identified via quantitative genetics and the TB risk that follows in their daughters. Consider in vitro or in vivo experiments to investigate how these extreme phenotypes handle the pathogen.

TB phenotypes, such as susceptibility and/or transmissibility may be expressed via structural genetic variation or via epigenetics.

M. bovis and macrophage gene expression (transcriptome/RNA sequence data) in different in vivo environments (macrophages from naïve and immune hosts)

Compare response where macrophages are infected with different *M. bovis* strains, BCG and *M. tuberculosis* looking at gene responses of the macrophage and the bacterium.

Compare response where neutrophils (and subsets including regulatory neutrophils) are infected with different *M. bovis* strains, BCG and M. tuberculosis looking at gene responses of the macrophage and the bacterium

Comparison of the macrophage-bacteria response following clearance (is infection ever cleared or just walled off?), in latency (this may involve comparative studies involving different breeds or species) and in active infections.
Comparison of the response to different strains of *M. bovis*
Incubation period in species other than cattle
Role of co-infections in disease progression.
Relationship between granuloma characteristics and the number of organisms present.
Establishing the role of γδT cells and granulocytes in granulomas.
Consider other important cell types such as different neutrophil subsets in the evolution of the disease

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]

Title:	Antigen identification

Research Question

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

To identify which *M. bovis* antigens would be most suitable for inclusion in tests based on humoral and/or CMI responses.

Challenge(s)

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

The host may be responding to different *M. bovis* antigens at different stages of infections.

M. bovis may be expressing different antigens at different stages, i.e., exposure, infection, reactivation, latency etc.

Antigens comprise peptides and proteins, including post-translational modifications such as glycosylation and acylation.

Solution Routes

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

Antigen mining based on pathogen genome sequence - Identification of T-cell antigens and epitopes using available bioinformatics algorithms and machine learning.

Comparative genomics of *M bovis* and other mycobacteria to identify markers for new diagnostics.

Identification of different antigens that are expressed by the pathogen during infection.

Immunoblotting using sera from infected animals.

Gene sequences being expressed in vivo at different stages of infection.

Most focus has understandably been on proteins and peptides; lipids and modified proteins likely to be important in host-pathogen interactions and TB lifecycle.

10% of the *M. bovis* genome comprises PE and PPE proteins; their role not clear.

Dependencies

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

HPI - Identification of which pathogen genes are being expressed in different stages of infection

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary [9]

Title:	Genome Characterisation (WGS)

Research Question

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

To exploit the *M. bovis* whole-genome sequence to identify antigens with diagnostic potential.

Challenge(s)

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

Challenge to understand how TB benefits from major antigens being highly conserved and under purifying selection.
Relatively limited genome diversity within the major *M. bovis* clonal complexes; some are fixed by location.
Development of standardised nomenclature linking MLVA(Multiple-Locus Variable number tandem repeat Analysis) and spoligotyping with Whole Genome Sequencing data.
Whole-genome sequencing provides unprecedented resolution; MLVA and spoligo data are highly correlated with it, although rare homoplasy evident.

Solution Routes

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

Ongoing genome annotation, identification of ORFs, gene expression and regulation (epigenetics), post-translational modification etc.
Comparative genomics within the MTBC (Mycobacterium Tuberculosis Complex) and beyond.
Bioinformatics T- and B-cell epitope mapping.
Structure and function prediction.
Identification of potential DIVA (Differentiation of Infected from Vaccinated Animals) diagnostic reagents.
Identification of molecularly defined PPDs.
Comparative genomics to investigate and control cross-reactivity.

Dependencies

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

State of the Art

Existing knowledge including successes and failures

A 24-loci MIRU-VNTR (Mycobacterial Interspersed Repetitive Units - Variable Number Tandem Repeat)genotyping is the gold standard for human TB diagnosis.
Reference and research labs increasingly transitioning to whole-genome-enabled approaches.

Projects *What activities are planned or underway?*

Lead Summary [10]

Title:	The detection of pathogen using molecular techniques

Research Question

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

The detection of the pathogen genome as a marker of infection.

Challenge(s)

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

Lack of accepted gold standard or Composite Reference Standard.
The most appropriate sample to use.
Efficient release of the organism from its intracellular location.
Avoidance/removal of amplification inhibitors.
Quality assurance - test repeatability and reproducibility.
Cost effectiveness.
Detection in biological samples including milk and faeces.
The most suitable primers and platform.
Risk of not having a pan-*M. bovis* gene sequences.

Solution Routes

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

Objective evaluation of candidate methods on reference samples.
Consider latent class analyses.
Cepheid GeneXpert and Ultra used in human TB reference labs.
Follow STARD and QUADAS-2 guidelines for reporting and designing diagnostic accuracy studies.

Dependencies

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

Sequencing of a range of *M. bovis* isolates from different territories and areas.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary [11]

Title:	Direct detection

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

10 above refers.

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

Culture time
Detection in biological samples including milk and faeces.
Improved culture media
Sample concentration

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

Development of enrichment broth

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 [11b]

Title:	Phage typing

Research Question

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

Evaluation of phage-based direct *M. bovis* detection.

Challenge(s)

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

Culture time.
Detection in biological samples including faeces.
Improved culture media.
Sample concentration.
Performing phage typing requires specialized technical expertise and facilities.
Time and labor Intensive.
Interpretation and standardization.
Limited discriminatory power.

Solution Routes

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

Objective evaluation of candidate methods on reference samples.
Consider latent class analyses.
Follow STARD and QUADAS-2 guidelines for reporting and designing diagnostic accuracy studies.
Development of enrichment broth.

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 [12]

Title:	Biomarkers

Research Question

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

To identify and validate specific molecules, indicators, or signatures that can serve as measurable indicators of infection, disease progression, treatment response, and disease control.
To develop biosignatures (combination of biomarkers) that could inform on the latent/carrier status of an animal and evaluate the risk of Mb shedding and transmission.

Challenge(s)

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

Current tests are based on direct detection and/or host immune responses.
Lack of accepted gold standard or Composite Reference Standard.
Identification of pathogen derived markers (metabolic products) and/or host factors that are not dependent on the host's immune response but are specific for infection.
Heterogeneity: host immune response to bTB can vary among individuals and even within the same individual over time. This heterogeneity poses a challenge in identifying consistent and reliable biomarkers that can accurately represent the diverse responses seen in different hosts.
M. bovis strains exhibit genetic diversity
Standardising protocols and validation criteria for biomarker detection assays is necessary to ensure consistent and reproducible results across different laboratories and settings.
Harmonisation of methodologies is essential to facilitate the comparability and reliability of biomarker-based diagnostic tests.
Ensuring the stability and integrity of biomarkers during sample collection, storage, and transportation.
To understand the different clinical states of TB in cattle akin to what is now established for human TB
To develop easy and affordable tools to identify biosignatures
To identify a biosignature in circulating blood (the easiest and less invasive procedures for sampling large numbers of animals)

Solution Routes

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

Detection of non-protein volatile components in air samples with mass spec.
Biomarkers can also play a role in vaccine development and evaluation by serving as indicators of vaccine effectiveness,

helping to identify protective immune responses, and aiding in the selection and monitoring of vaccine candidates.
Consider latent class analyses.
Follow STARD and QUADAS-2 guidelines for reporting and designing diagnostic accuracy studies.
To embrace the One Health approach and work together with human TB specialists

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

To establish robust pipelines with all stakeholders-including bTB surveillance programs- to ensure rigorous and longitudinal follow-up of animals in the field

State of the Art
Existing knowledge including successes and failures

Projects
What activities are planned or underway?

Lead Summary [Number]

Title:	

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 [Number]

Title:	

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?