



STO TECHNICAL REPORT

TR-HFM-230

Development of a Depository of Fast and Reliable Detection Methods for Vector-Borne and Zoonotic Pathogens

(Elaboration d'un catalogue de méthodes rapides
et fiables de détection des agents pathogènes
zoonotiques et transmis par vecteur)

Final Report of HFM-230.



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The NATO Science and Technology Organization

Science & Technology (S&T) in the NATO context is defined as the selective and rigorous generation and application of state-of-the-art, validated knowledge for defence and security purposes. S&T activities embrace scientific research, technology development, transition, application and field-testing, experimentation and a range of related scientific activities that include systems engineering, operational research and analysis, synthesis, integration and validation of knowledge derived through the scientific method.

In NATO, S&T is addressed using different business models, namely a collaborative business model where NATO provides a forum where NATO Nations and partner Nations elect to use their national resources to define, conduct and promote cooperative research and information exchange, and secondly an in-house delivery business model where S&T activities are conducted in a NATO dedicated executive body, having its own personnel, capabilities and infrastructure.

The mission of the NATO Science & Technology Organization (STO) is to help position the Nations' and NATO's S&T investments as a strategic enabler of the knowledge and technology advantage for the defence and security posture of NATO Nations and partner Nations, by conducting and promoting S&T activities that augment and leverage the capabilities and programmes of the Alliance, of the NATO Nations and the partner Nations, in support of NATO's objectives, and contributing to NATO's ability to enable and influence security and defence related capability development and threat mitigation in NATO Nations and partner Nations, in accordance with NATO policies.

The total spectrum of this collaborative effort is addressed by six Technical Panels who manage a wide range of scientific research activities, a Group specialising in modelling and simulation, plus a Committee dedicated to supporting the information management needs of the organization.

- AVT Applied Vehicle Technology Panel
- HFM Human Factors and Medicine Panel
- IST Information Systems Technology Panel
- NMSG NATO Modelling and Simulation Group
- SAS System Analysis and Studies Panel
- SCI Systems Concepts and Integration Panel
- SET Sensors and Electronics Technology Panel

These Panels and Group are the power-house of the collaborative model and are made up of national representatives as well as recognised world-class scientists, engineers and information specialists. In addition to providing critical technical oversight, they also provide a communication link to military users and other NATO bodies.

The scientific and technological work is carried out by Technical Teams, created under one or more of these eight bodies, for specific research activities which have a defined duration. These research activities can take a variety of forms, including Task Groups, Workshops, Symposia, Specialists' Meetings, Lecture Series and Technical Courses.

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List of Abbreviations and Acronyms

AMedP	Allied Medical Publication
ASTER	Alerte et surveillance en temps réel
BEL	Belgium
BSC	Biosafety Cabinet
BSL	Biosafety Level
CDC	Centre for Disease Control
CE	Conformité Européene
COMEDS	Committee of the Chiefs of Military Medical Services in NATO
CZE	Czech Republic
DEU	Germany
ECDC	European Centre for Disease Control
EHEC	Enterohemorrhagic Escherichia Coli
EID	Emerging Infectious Diseases
ELISA	Enzyme-Linked Immunosorbent Assay
ENIVD	European Network for Diagnostics of “Imported” Viral Diseases
EPINATO	NATO epidemiological data collection system
ET	Exploratory Team
ETEC	Enterotoxigenic Escherichia Coli
FAO	Food and Agricultural Organisation
FDA	Food and Drug Administration
FHP	Force Health Protection
FRA	France
GMT	Good Microbiological Techniques
HFM	Human Factors and Medicine (Panel)
HIV	Human Immunodeficiency Virus
HTLV	Human T-Cell Leukemia Virus
IATA	International Air Transport Association
ITA	Italy
LAMP	Loop-mediated isothermal amplification
MEDINT	Medical Intelligence
MERS	Middle Eastern Respiratory Syndrome
MRB	Multi-Resistant Bacteria
MRSA	Multi-Resistant Staphylococcus Aureus
NA	Nucleic Acids
NASBA	Nucleic Acid Sequence-Based Amplification
NATO	North Atlantic Treaty Organization
PAO	Pan African Organisation
PCR	Polymerase Chain Reaction

PoW	Programme of Work
PrevMed	Preventive Medicine
PSDS	Pathogen Safety Data Sheet
OIE	World Animal Health Organisation (Office International des Epizooties)
ROU	Romania
RTG	Research Task Group
STANAG	Standardized NATO Agreement
SurSaUD	Surveillance Sanitaire des Urgences et des Décès
SVN	Slovenia
TAD	Technical Activity Description
TAP	Technical Activity Proposal
ToR	Terms of Reference
TUR	Turkey
UN	United Nations
USA	United States of America
WHO	World Health Organisation
WWII	Second World War

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We would like to recognize the time and effort put into the priority scoring of the different pathogens as well as compiling the list of state of the art detection methods, done by specialists in each member nation of this RTG.

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As a conclusion, we hope that the output of this working group will benefit to all NATO member states, in order to optimise diagnosis of zoonotic and vector-borne diseases with impact on the military strength in operational settings, to allow for surveillance and ultimately contribute to Force Health Protection worldwide.

In a world where detection methods evolve at a very fast pace, it is clear that this work is not done; as a final remark, we hope that this project can continue to support the health and well-being of our troops.

Respectfully,

//SIGNED//

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In Memory of Christian Vandenvelde

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Development of a Depository of Fast and Reliable Detection Methods for Vector-Borne and Zoonotic Pathogens (STO-TR-HFM-230)

Executive Summary

The NATO Human Factors and Medicine Research Task Group 230, “Development of a Depository of Fast and Reliable Detection Methods for Vector-Borne and Zoonotic Pathogens”, worked from 2012 to 2016. It was founded in order to summarize the scientific knowledge on the military impact of pathogens that are transmitted from animals to man either by having an animal reservoir and/or vector such as insects, ticks etc. The RTG included over 17 representatives from 9 countries, was headed by Belgium and included partners from the Czech Republic, France, Germany, Italy, Romania, Slovenia, Turkey and the USA.

In summary, the RTG HFM-230 had two preparative meetings that were held in 2011, and eight meetings between 2012 and 2016 during which the group had intensive discussions and worked on the comprehensive literature and data collections (Chapter 2).

One goal of RTG HFM-230 was to develop priority lists (Chapter 3) for vector-borne and zoonotic bacteria, parasites and viruses which was accomplished via a comprehensive literature review and input from RTG country representatives. These priority lists were combined to one final master priority list. The group also summarized information on biosafety levels (Chapter 4) and sample collection and transport (Chapter 5). An additional goal was to do an intensive review of available field and laboratory detection methods for the previously prioritized viruses, bacteria and parasites. As missions differ in their requirements for and ability to support the various diagnostic capabilities, it was decided to differentiate between first and second line detection methods. First line detection ‘in the field’ is more easily implemented but may require well trained and experienced personnel if microscopy is utilized. Second line detection ‘in the lab’ enables more advanced diagnostic testing. In the event more advanced diagnostic testing is required, specific infrastructure and specialized personnel are needed (Chapter 6). Commercial as well as literature-based assays were reviewed and listed, also information was provided on the pathogen, matrix of samples, and – if available – recommendations on the use of the respective assay. Finally the group summarized the presently available civil and military surveillance systems (Chapter 7). A gap analysis and recommendations concerning these five goals are offered in Chapters 8 and 9.

The RTG consisted of highly motivated and collaborative country representatives that shared their knowledge as well as their different and broad expertise, to include virologists, bacteriologists, parasitologists, entomologists, epidemiologists, veterinarians and infectious disease physicians.

A major outcome of the RTG HFM-230 was the comprehensive collection of detection methods for the selected important zoonotic and vector-borne pathogens. The work of the RTG HFM-230 members is a reflection of efforts from 2012 to 2016 and is a living document that will require on-going revision by a future RTG as new diseases emerge and technology improves.

History has repeatedly demonstrated the importance of force health protection in military operations. Access to established surveillance systems such as EPINATO is necessary for medical personnel to make informed recommendations to the chain of command and likewise to support Commanders decisions. In addition,

the reliance on and the confidence in different laboratory systems used by the various NATO member countries calls for a standardized platform whereby member laboratories can share data, discuss diagnostic protocols and allow for scientific exchange. Therefore the development and installation of a laboratory network for near real-time surveillance is strongly recommended.

Elaboration d'un catalogue de méthodes rapides et fiables de détection des agents pathogènes zoonotiques et transmis par vecteur

(STO-TR-HFM-230)

Synthèse

Le groupe de recherche 230 de la Commission sur les facteurs humains et la médecine de l'OTAN, intitulé « Elaboration d'un catalogue de méthodes rapides et fiables de détection des agents pathogènes zoonotiques et transmis par vecteur », a travaillé de 2012 à 2016. Ce groupe de recherche avait été créé pour résumer les connaissances scientifiques sur l'effet militaire des agents pathogènes transmis à l'homme par des animaux, soit par le biais d'un réservoir animal, soit par le biais de vecteurs tels que des insectes, des tiques, etc. Présidé par la Belgique, le RTG se composait de plus de 17 représentants de neuf pays, à savoir, outre la Belgique, la République tchèque, la France, l'Allemagne, l'Italie, la Roumanie, la Slovaquie, la Turquie et les États-Unis.

En résumé, le RTG HFM-230 a tenu deux réunions préparatoires en 2011, puis huit réunions entre 2012 et 2016, au cours desquelles le groupe a mené des discussions intensives et a travaillé sur la littérature complète et le recueil des données (chapitre 2).

L'un des objectifs du RTG HFM-230 était d'établir des listes de priorités (chapitre 3) en matière de bactéries, parasites et virus zoonotiques et transmis par vecteur, ce qui a été réalisé au moyen d'une revue complète de la littérature et d'apports des participants du RTG. Ces listes de priorités ont été combinées en une liste principale définitive des priorités. Le groupe a également résumé les informations sur les niveaux de biosécurité (chapitre 4) et le recueil et le transport d'échantillons (chapitre 5). Un autre objectif consistait à mener une revue intensive des méthodes de détection disponibles sur le terrain et en laboratoire pour les virus, bactéries et parasites prioritaires. Étant donné que les besoins et la capacité de soutien des diverses méthodes diagnostiques varient selon les missions, il a été décidé de faire la différence entre les méthodes de détection majeures et secondaires. Les méthodes de détection majeures « sur le terrain » sont plus faciles à appliquer, mais peuvent exiger du personnel expérimenté et qualifié si la microscopie est utilisée. Les méthodes de détection secondaires « en laboratoire » permettent des essais diagnostiques plus poussés. Lorsque des essais diagnostiques plus poussés sont nécessaires, des infrastructures spécifiques et du personnel spécialisé sont nécessaires (chapitre 6). Les essais commerciaux et basés sur la littérature ont été examinés et répertoriés. Des informations ont été fournies sur le pathogène et sur la grille des échantillons, ainsi que, le cas échéant, des recommandations sur l'utilisation de l'essai respectif. Enfin, le groupe a résumé les systèmes de surveillance civils et militaires actuellement disponibles (chapitre 7). Les chapitres 8 et 9 fournissent une analyse des écarts et des recommandations concernant ces cinq objectifs.

Le RTG se composait de représentants nationaux extrêmement motivés et collaboratifs, qui ont partagé leurs connaissances et leur vaste expertise dans différents domaines (virologie, bactériologie, parasitologie, entomologie, épidémiologie, médecine vétérinaire et maladies infectieuses).

L'un des grands résultats du RTG HFM-230 a été le recueil de toutes les méthodes de détection des agents pathogènes importants, zoonotiques et transmis par vecteur. Le rapport des membres du RTG HFM-230 reflète les travaux menés de 2012 à 2016. Il s'agit d'un document évolutif qui devra être révisé à l'avenir, avec l'apparition de nouvelles maladies et l'évolution des technologies.

L'histoire a prouvé à plusieurs reprises l'importance de la protection sanitaire des forces armées en opération. L'accès à des systèmes de surveillance établis tels qu'EPINATO est nécessaire pour que le personnel médical puisse émettre des recommandations éclairées à l'usage de la chaîne de commandement et faciliter les décisions des commandants. De plus, la fiabilité et la dépendance à l'égard des différents systèmes de laboratoire utilisés dans les différents pays de l'OTAN nécessitent une plateforme normalisée sur laquelle les laboratoires membres puissent partager leurs données, discuter des protocoles de diagnostic et échanger sur le plan scientifique. Par conséquent, il est fortement recommandé de développer et de mettre en place un réseau de laboratoires consacré à une surveillance proche du temps réel.

Chapter 1 – INTRODUCTION

Bacterial, parasitic and viral zoonoses have had their influence on military campaigns throughout history, at times playing an important role in determining the result. As infectious diseases have the ability to significantly influence military capabilities and options, the availability of approved diagnostics or detection methods and near-real time surveillance data to inform the chain of command is of high importance for military missions.

To date more than 1400 pathogens can affect humans, of these more than 60% are zoonotic. Among these pathogens, “Emerging Infectious Diseases” (EID) serve as a special risk especially in mission areas where soldiers are a vulnerable population. At least 75% of EID are zoonotic, pointing to animal reservoirs and vectors as the primary source of infection. Sentinel animals can serve as an early warning system if pathogens are detected before human illness emerges, at which time preventive measures can be enacted. In addition migrating birds, commercially shipped livestock, domesticated and wild animals as well as pet trade play a major role in spreading and maintaining an epidemic over large distances. Transportation of zoonotic pathogens by means of infected personnel, animals or inanimate objects (e.g., vehicles, food, souvenirs) from a theatre of operation to the home country (or vice versa) could generate an outbreak.

Therefore the RTG HFM-230 was established to:

“Define standards for first line (“in the field”) and second line (“in the lab”) detection and identification methods for viral and bacterial zoonoses. The RTG will also review the outbreak alert system and on-sight surveillance and create a list of pathogen collections of which items can serve as positive control or for validation purposes.”

During the RTG meetings, parasitic diseases were included due to their high presence in tropical and subtropical regions.

1.1 ORIGINS OF RTG HFM-230

In 2005, Paul Heyman (BEL) and PD Dr. Sandra Essbauer (DEU) collaborated in a civilian “European network of imported viral diseases”, a platform for scientists from university medical centres, country health departments, and hospitals. Recent outbreaks of viral hemorrhagic fevers such as ebola virus and hantavirus as well as bacteria such as Enterohemorrhagic Escherichia coli (EHEC) in areas of operation or training, reinforce the importance of maintaining an in depth knowledge of these pathogens. As three-fourths of emerging infections are transmitted from animals to man (are zoonotic in nature), military personnel in training or on missions are at particular risk during daily operations.

Med OF-6 Pierre Neirinckx (BEL), member of the NATO Human Factors and Medicine panel, supported the idea of creating a working group on zoonotic diseases.

The Exploratory Team (ET-118) was initiated by Med OF-4 Christian Vandenvelde and Paul Heyman (BEL) in 2011 to generate a NATO network to collect data on emerging and re-emerging zoonotic and arthropod-borne infections of military relevance for NATO countries. In accordance with the One Health Concept, specialists like physicians, veterinarians and biologists were invited to take part in such a group.

Belgium, Germany, France, the USA, Slovenia, Romania, Italy, Czech Republic and Turkey agreed to participate in the ET. This ET-118 was accepted in August 2011 at the Halifax meeting.

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ET-118 resulted in a Technical Activity Proposal (TAP) (Annex A), Terms of Reference (ToR) (Annex B), a Program of Work (PoW) (Annex C) and led to the beginning of RTG HFM-230 in May 2012.

1.2 OBJECTIVES OF RTG HFM-230

1.2.1 Original Objectives

The original objectives for a Research Task Group (RTG) were established during the two meetings of ET-118 (summarized in Chapter 2).

The first objective was to define standards for first line (“in the field”) and second line (“in the lab”) detection and identification methods for viral and bacterial zoonoses.

A second objective was to review the outbreak alert system and on-sight surveillance, and create a list of pathogen collections of which items can serve as positive control or for validation purposes.

The topics to be covered were the following:

- Depository of fast, reliable detection and identification methods for viral zoonoses.
- Depository of fast, reliable detection and identification methods for bacterial zoonoses.
- Charting existing pathogen collections / defining priority pathogens.
- Outbreak alert communication.
- On-sight surveillance of priority pathogens.

The Group decided on the following deliverables and end product:

- Annual reports.
- Technical reports (final RTG report).
- Reviews of the literature to be published in peer-reviewed (military) scientific journals.
- Presentation of the work during a NATO symposium.
- Presentation of the work during an International symposium.

1.2.2 Final Objectives

During the different meetings the original objectives were slightly changed and other objectives were added. Early on in the initial ET-118 meeting, the addition of parasites to the viruses and bacteria was agreed upon, as well as adding vector-borne pathogens.

Closely related to the topic of prioritization, the working group decided to perform an extensive literature search on selected keywords in order to include all pathogens that have been a problem for the health of troops worldwide.

Charting the existing pathogen collections has been discussed, but is impossible at this moment because of the lack of a NATO-wide laboratory network.

In 2012 a part of the NATO panel working on hantaviruses summarized the current knowledge on hantaviruses in Europe in a publication in “International Review of the Armed Forces Medical Services”. [24]

Presentation during the XVth Biodefense Conference in Munich, Germany (26-29 April 2016).

Presentation during the COMEDS meeting in Potsdam, Germany (September 2016).

Presentation during the 47th Annual Congress of the German Medical Society (Deutsche Gesellschaft für Wehrmedizin und Wehrpharmazie e. V.) in Ulm, Germany (05-08 October 2016).

1.3 INTENDED USE OF THIS DOCUMENT

This document is the result of the work of nine NATO member countries and may not be representative for all NATO countries. It is intended to be used as a tool, by which NATO members may identify resources and fill gaps in an individual country’s armamentarium of laboratory capabilities. It may be used as a launching point for discussions concerning pathogen and detection priorities and provides a forum and reference for future exchange between NATO members.

This document is not intended to be an all-encompassing resource for detection methods.

Nor is it intended to be a medical reference for diagnosis and should not replace consultation with the NATO member’s clinical and research laboratory experts. Knowledge and advancements in diagnostic and detection capabilities are continually developing and members are encouraged to verify the most current standards.

1.4 FIRST LINE AND SECOND LINE DETECTION

Infectious diseases are of major concern in military missions abroad, especially if missions take place in less-developed countries or areas. Human infections with zoonotic and/or vector-borne pathogens occur worldwide, often in areas of operation where there is limited knowledge on prevalence, reservoirs, vectors and actual epidemiological data.

Commanders must make decisions on medical and diagnostic support utilizing initial information, which is often difficult to verify, from medical intelligence activities.

Decisions concerning diagnostic capabilities, to include numbers of personnel, equipment and infrastructure, during military operations are affected by political, strategic and tactical considerations. Hence, the decision concerning extent, readiness and accuracy of diagnostic capabilities in mission has to focus on infectious diseases prevalent or possible in the area of operation and how they affect or impede the mission’s task.

Depending on the military setting different diagnostic approaches have to be evaluated. If diagnostic capabilities are necessary but only limited extent of experienced personnel is available, the “diagnostics in the field” might be a solution although sometimes with limited diagnostic quality. If diseases of concern require more advanced diagnostic techniques, than “diagnostics in the lab” may need to be provided.

First “line detection methods” can be done without specific infrastructure, e.g., inside tents or vehicles. Experienced, trained personnel are needed and possibly a power source (battery or small generator) depending on the type of testing required. The “first line detection methods” should be easy to handle, ready to use and very

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robust concerning transport and storage. For operational issues, these methods may enable at least a hint for the pathogen in apparently ill persons. In contrast to this, the “second line detection methods” need specific infrastructure like deployable laboratory containers or a building with a laboratory or glove box, preferentially at least Biosafety level 2. To operate such a laboratory, experienced, trained personnel is required. These detection methods may require more advanced storage capabilities (e.g., chilling, freezing), equipment (e.g., biological safety cabinet, ELISA-reader, fluorescence microscope, incubator, NA-preparation accessories, thermal cycler, etc.) and infrastructural capabilities (e.g., air-conditioning, drinking and waste water connection, electricity, air filtering). The methods used are as sensitive and specific as possible. In summary, the algorithm for detection of pathogens therefore in general starts with simple assays in the field laboratory; followed by further confirmatory assays that might also be performed in the field lab. However, further confirmatory assays may require a transfer of the samples to a stationary lab. Additional samples can be transported to expert labs to perform an in-depth characterization or final confirmation of the pathogen as required in some cases, e.g., for serological differentiation of cross-reactive flavi-, bunyaviruses.

Chapter 2 – OVERVIEW OF THE MEETINGS

2.1 INTRODUCTION

The main issues raised and discussed during each meeting are described in this chapter. Every chapter contains a short explanation of the topics discussed and the actions coming from each meeting.

2.2 ET-118 PREPARATORY MEETING BRUSSELS, BELGIUM (11-14 JULY 2011)

Participating Countries: BEL, DEU, FRA, SVN, TUR, USA.

The first ET-118 meeting started with a formal introductory presentation of each delegate concerning their institute and their work.

Med OF-6 Pierre Neirinckx, HFM Panel Member and country representative (BEL), welcomed the participants and gave instructions concerning the lead and the set-up of an ET and RTG group.

During the meeting the objectives and the targets of a future RTG were discussed.

The first draft of TAP, ToR, PoW was prepared with collaboration of all participants.

ET-118 was accepted in August 2011.

2.3 ET-118 PREPARATORY MEETING MARSEILLE, FRANCE (5-8 DECEMBER 2011)

Participating Countries: BEL, DEU, FRA, ITA, SVN, TUR, USA.

Welcomed the new members OF-3 Leen Wilmaerts (BEL) and Med OF-5 Florigio Lista (ITA), both of whom gave a presentation on their institute and their work.

The French mobile laboratory concept was presented.

The parasitology and the virology laboratory were visited.

The drafts of the TAP, ToR and PoW were discussed.

Early on in the discussions, it was decided to develop a priority list of pathogens to include not only bacteria and viruses, but also parasites. The scope of zoonotic pathogens was broadened to include vector-borne pathogens. A first list of pathogens to score for priority was made based on relevant NATO documents.

The existence of other pathogen priority lists within other NATO documents (e.g., STANAG) and whether they need to be included was discussed.

The TAP, ToR and Pow were finalized.

Belgium volunteered to develop a methodology to prioritize the pathogens (criteria definition, scoring and pathogens) for the next meeting.

2.4 RTG HFM-230 MEETING #1 NEUILLY-SUR-SEINE, FRANCE (29 MAY – 1 JUNE 2012)

Participating Countries: BEL, CZE, DEU, FRA, ITA, USA.

Welcomed new members OF-3 Vera Neubauerova (CZE), OF-3 Clayton Chilcoat (USA), who replaced OF-3 Robert Webb, and OF-5 Michael Faulde (DEU), who provided presentations on their institute and work.

An overview of the RTG guidelines was given by OF-4 RNLA Ron Verkerk (HFM Panel Executive, NLD).

Amendments to the TAP, ToR, and PoW were reviewed and discussed by Ron Verkerk. The TAP, ToR, PoW were finalized and submitted.

A priority list tool (criteria definitions and scorings as well as a top 10 priority list for each group of pathogens) was proposed by Christian Vandenvelde. The proposal resulted in a discussion and different presentations on methodologies for prioritization.

The conceptual structure of the proposed priority lists (criteria, scoring and top 10 pathogens) was agreed upon by the members. Each RTG member country was requested to submit a priority list of pathogens from their individual country perspective utilizing the proposed priority tool.

2.5 RTG HFM-230 MEETING #2 ROME, ITALY (17-20 DECEMBER 2012)

Participating Countries: BEL, CZE, DEU, FRA, ITA, SVN, USA.

Welcomed the new member Giovanni Faggioni (ITA), who gave a presentation on his institute and work.

Presentations on the surveillance and on the detection methods of some pathogens were given by the host NATO country.

The priority list and the detection methods of the three groups of pathogens were discussed. In order to make the list more relevant, it was decided to develop a list of the top 25 pathogens.

It was agreed upon to perform a literature search on zoonotic and vector-borne diseases that have affected militaries since WWII. Keywords were defined for this search.

The meeting in Rome provided necessary information concerning the priority pathogen templates.

A deadline for submission was set for the priority lists per pathogen type (bacteria, viruses, parasites). A presentation of our priority list was given at the BioMedac forum on 16 May 2012.

2.6 RTG HFM-230 MEETING #3 LJUBLJANA, SLOVENIA (30 SEPTEMBER – 5 OCTOBER 2013)

Participating countries: BEL, CZE, DEU, FRA, ROU, SVN.

Welcomed new member, MSc. Diana Popescu (ROU), who gave a presentation on her institute and work.

The Slovenian Veterinary unit was presented and a tour of the laboratory was provided.

Presentations were given by the members of our NATO group and by invited speakers.

The KRKA Company (specializing in medical supplies and disinfection) and the BSL4 laboratory of Prof. Tatajana Avšič - Županc, BSc (Biol), PhD were visited.

The literature search produced a substantial number of articles. The resultant extensive review of the literature was divided into the three pathogen groups, corresponding to the three pathogen (viruses, bacteria, parasites) groups. The group also agreed upon a template for the literature search.

The search for Pathogen Safety Data Sheets (PSDS) files was also divided into three pathogen groups.

The meeting in Ljubljana was highly successful and productive. It lasted 4 days instead of the usual 3 in order to make up for the time lost as a result of the cancelled spring meeting in 2013.

The preliminary priority lists were agreed upon waiting the additional pathogens following the literature search.

A deadline for submission to the lead was set for the reading of the literature search.

2.7 RTG HFM-230 MEETING #4 ROME, ITALY (24-28 MARCH 2014)

Participating countries: BEL, CZE, DEU, FRA, ITA, ROU, SVN, USA.

Two new chairmen were appointed: MSc. Christel Cochez and OF-3 Leen Wilmaerts (both from Belgium).

Welcomed new member, OF-4 Pamela Ward-Demo (USA), as a replacement of OF-4 Clayton Chilcoat due to his reassignment. The new member gave a presentation on her institute and work.

The literature search results were discussed and finalized. The additional pathogens found in the literature search were added to the theoretical pathogen priority lists. The additional pathogens were scored as a consensus by the members present during this meeting.

It was decided to provide only an “anonymous” general priority list from each of the member countries in the final report. No individual country scores will be published.

The decision was taken to use the PSDS files that are produced by Public Health Canada, as they are of outstanding quality and available online.

A discussion regarding the usefulness of priority pathogen lists was held.

The required details of the detection methods were discussed. It was decided to work in 3 groups (virus, bacteria and parasites) with a group leader. The template of the table was agreed upon.

Due to the extra work with the literature search and the changes in chairmen and other members, the possibility of asking for another year was discussed.

OVERVIEW OF THE MEETINGS

2.8 RTG HFM-230 MEETING #5 PRAGUE, CZECH REPUBLIC (24-28 NOVEMBER 2014)

Participating countries: BEL, CZE, DEU, FRA, SVN.

The Centre of Biological Defence in Techonin was visited.

The so far listed detection methods were reviewed and discussed.

The RTG members developed PSDSs for those pathogens without a PSDS available on line and publish these as an annex in the report.

During the meeting it was decided to provide detailed information concerning available field assays for diagnosis. In addition, the group determined that information concerning reference laboratories would be beneficial in case required for confirmatory testing, as well as information about RTG member experience with the various assays or other advanced methodologies.

It was determined to include an explanation of how the priority list was developed, rather than explaining the context of each pathogen, in the final report.

The template of the detection list table was changed in order to add more detail to meet the needs.

RTG HFM-230 was granted for one extra year.

2.9 RTG HFM-230 MEETING #6 GHENT, BELGIUM (4-8 MAY 2015)

Participating countries: BEL, CZE, DEU, FRA, USA.

Welcomed a new member, OF-4 Ulrich Schotte (DEU), who replaced Alfred Binder after his retirement.

The RTG members discussed details of the final report, to include content and formatting of the chapters and tables. The meeting included a discussion of the development of new and re-emerging diseases ('e.g., MERS, Zika) since the inception of the RTG group and as such were not considered during the development of the priority lists. The absence of these new and re-emerging pathogens in the priority list will be discussed in the gap analysis and noted as need for a follow-on group to continue this work. In addition the members noted the ranking of certain pathogens in the priority list did not seem plausible and alterations were made accordingly.

2.10 RTG HFM-230 MEETING #7 LJUBLJANA, SLOVENIA (7-11 SEPTEMBER 2015)

Participating countries: BEL, DEU, FRA, SVN, USA.

The template of the final report and user guidance was discussed.

The contents of the final report were decided upon, and the authors of the different chapters were designated.

The knowledge gaps were discussed.

2.11 RTG HFM-230 MEETING #8 MUNICH, GERMANY (23-26 FEBRUARY 2016)

Participating countries: BEL, DEU, FRA, ROU, SVN, USA.

The different laboratories of the Institute for Microbiology were visited.

The knowledge gaps and recommendations were further discussed.

The first draft of the final report was read and discussed, and writing was continued.

OVERVIEW OF THE MEETINGS



Chapter 3 – PATHOGENS PRIORITY LIST

3.1 INTRODUCTION

Many of today's known infectious pathogens are zoonotic or vector-borne. Taylor *et al.* [92] describe a list of at least 1415 pathogens infectious to humans, of which 61% are zoonotic. Amongst the pathogens considered as emerging, at least 75% are zoonotic. It would have been an impossible job to detail detection methods for each and every one of those.

Therefore one of the primary goals of this working group was to prioritize the known vector-borne and zoonotic pathogens with an emphasis on those which are of concern in a military setting. It is important to realise that any form of prioritization is only useful in the context in which it is conducted [43]. This is discussed further in this chapter.

In an effort to be as complete as possible, an extensive PUBMED literature search was completed on selected keywords. Aim of this work was to include all vector-borne and zoonotic pathogens of military concern, as an addition to the pathogens that immediately came to mind. The methodology and results for this performed task are also discussed in this chapter.

Priority lists were generated by the countries contributing to the RTG HFM-230. At the beginning, ten selected viruses, bacteria and parasites were identified. During the 4th meeting of RTG HFM-230 in Rome (Spring 2014), it was decided to not limit the priority list to the top 10 as this would result in important pathogens falling off the list. So the first lists were expanded gradually to include all zoonotic and vector-borne pathogens of military significance. Finally, the panel decided to prioritize 30 viruses, 27 bacteria and 24 parasites.

3.2 LITERATURE STUDY

3.2.1 Introduction

In order to obtain an in depth review of the extent and importance of zoonotic and vector-borne pathogens during military operations, an extensive literature review was accomplished.

3.2.2 Methodology

In order to collect the present knowledge on relevant zoonotic pathogens in the context of military action an in depth literature study was performed. The basic source was PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), which is an open access online database that contains over 25 million citations on biomedical literature including also MEDLINE, life science journals and online books. The results were cross-checked with lists on notifiable diseases from OIE, WHO and PAO.

Keywords selected for the combined search in PubMed were:

- Conflict;
- War;
- Military;
- Disease;

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- Infectious;
- Bacteria;
- Virus;
- Parasite;
- Zoonotic;
- Vector-borne;
- Outcome; and
- Burden.

3.2.3 Result

The literature research in PubMed resulted in 17,308 articles that were reviewed in order to receive an overview on military relevant zoonotic viruses, bacteria and parasites (Status of Search: 22 January 2013). The numbers of papers that were found by using selected keywords are summarized in Table 3-1.

Table 3-1: Number of Papers Found on PubMed with Selected Keywords.

Keyword 1	Keyword 2	Keyword 3	Number of Articles
Conflict	Infectious Disease	–	209
Conflict	Bacteria	–	490
Conflict	Virus	–	336
Conflict	Parasite	–	139
Conflict	Zoonotic	–	8
Conflict	Vector-borne	–	4
War	Infectious Disease	–	1340
War	Bacteria	–	2095
War	Virus	–	1193
War	Parasite	–	128
War	Zoonotic	–	73
War	Vector-borne	–	29
Military	Infectious Disease	–	1245
Military	Bacteria	–	4995
Military	Virus	–	3663
Military	Parasite	–	314
Military	Zoonotic	–	68
Military	Vector-borne	–	60

Keyword 1	Keyword 2	Keyword 3	Number of Articles
Conflict	Infectious Disease	Outcome	9
Conflict	Bacteria	Outcome	25
Conflict	Virus	Outcome	17
Conflict	Parasite	Outcome	7
Conflict	Zoonotic	Outcome	0
Conflict	Vector-borne	Outcome	0
War	Infectious Disease	Outcome	38
War	Bacteria	Outcome	84
War	Virus	Outcome	37
War	Parasite	Outcome	4
War	Zoonotic	Outcome	1
War	Vector-borne	Outcome	1
Military	Infectious Disease	Outcome	82
Military	Bacteria	Outcome	249
Military	Virus	Outcome	144
Military	Parasite	Outcome	16
Military	Zoonotic	Outcome	4
Military	Vector-borne	Outcome	1
Conflict	Infectious Disease	Burden	14
Conflict	Bacteria	Burden	9
Conflict	Virus	Burden	4
Conflict	Parasite	Burden	7
Conflict	Zoonotic	Burden	1
Conflict	Vector-borne	Burden	0
War	Infectious Disease	Burden	31
War	Bacteria	Burden	26
War	Virus	Burden	16
War	Parasite	Burden	3
War	Zoonotic	Burden	2
War	Vector-borne	Burden	4

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Keyword 1	Keyword 2	Keyword 3	Number of Articles
Military	Infectious Disease	Burden	18
Military	Bacteria	Burden	34
Military	Virus	Burden	22
Military	Parasite	Burden	5
Military	Zoonotic	Burden	3
Military	Vector-borne	Burden	1

1279 articles included the keyword “conflict”, 10924 articles the keyword “military” and 5105 the keyword “war”. The keyword “burden” was found in 200 papers and “outcome” in 719 articles.

In summary, abstracts of 2986 articles were screened that included “infectious disease”, 200 the keyword “vector-borne”, 8007 with “bacteria”, 623 with “parasites”, 5410 with “virus” and 160 with the keyword “zoonotic”.

From these articles, a list of pathogens with outbreaks during military missions was gathered with information on involved nations, date and region of the outbreak, case numbers, case fatality, countermeasures, confirmatory methodology and civilian involvement. The list includes 48 viruses, 211 bacteria and 75 parasites. Pathogens that were not zoonotic or vector-borne were excluded.

The following tables give a brief overview of the respective genera or species that finally were relevant (Table 3-2 to Table 3-4).

Table 3-2: Summary Excerpt of Relevant Articles on Zoonotic and/or Vector-Borne Viruses Associated with Military Missions by PubMed Research (Status of Search: 22 January 2013).

Virus	Number of Found Relevant Articles
Hantavirus	7
Adeno virus	3
Influenza virus	3
Lassa virus	2
Rabies virus	2
Sandfly fever virus	2
Alkhurma virus	1
Calicivirus	1
Crimean-Congo hemorrhagic fever virus	1
Dengue virus	1
Coronavirus	1
Marburg virus	1

Table 3-3: Summary Excerpt of Relevant Articles on Zoonotic and/or Vector-Borne Bacteria Associated with Military Missions by PubMed Research (Status of Search: 22 January 2013).

Bacteria	Number of Found Relevant Articles
<i>Bacillus anthracis</i>	23
<i>Coxiella burnetii</i>	17
<i>Mycobacterium tuberculosis</i>	9
<i>Campylobacter</i>	8
<i>Leptospira</i>	8
<i>Francisella tularensis</i>	7
MRSA	7
<i>Brucella sp.</i>	6
<i>Escherichia coli</i> / Enterotoxigenic /ETEC	5
<i>E. coli</i> , <i>Shigella</i>	4
<i>Burkholderia pseudomallei</i>	3
<i>Brucella melitensis</i>	2
<i>Rickettsia prowazekii</i>	2
<i>Salmonella typhi</i>	2
<i>Staphylococcus aureus</i>	2
<i>Anaplasma</i> , <i>Ehrlichia</i> , <i>Bartonella</i>	1
<i>B. anthracis</i> , <i>Clostridium botulinum</i> , Norovirus	1
<i>Bacillus anthracis</i> , <i>Clostridium botulinum</i> , <i>Brucella sp.</i> , <i>Vibrio cholerae</i> , <i>Yersinia pestis</i> , <i>Francisella tularensis</i>	1
<i>Bacillus anthracis</i> , <i>Francisella tularensis</i>	1
<i>Bacillus anthracis</i> , <i>Yersinia pestis</i>	1
<i>Borrelia recurrentis</i>	1
<i>Brucella</i>	1
<i>Burkholderia mallei</i>	1
<i>Campylobacter</i> , <i>Shigella</i> , Norovirus	1
<i>Escherichia coli</i> , <i>Plesiomonas shigelloides</i> , <i>Salmonella</i> , <i>Shigella</i> , <i>Aeromonas</i> , <i>Vibrio</i>	1
MRB MRSA, <i>Acinetobacter</i> , <i>Pseudomonas</i>	1
MRB, <i>Staphylococcus aureus</i>	1

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Bacteria	Number of Found Relevant Articles
<i>Mycobacterium</i> , drug res	1
<i>Neisseria meningitidis</i> , <i>Mycoplasma pneumoniae</i> , <i>Brucella melitensis</i> , <i>Salmonella typhi</i>	1
<i>Orientia tsutsugamushi</i>	1
<i>Rickettsia</i>	1
<i>Rickettsia prowazekii</i> , <i>Baronella quintana</i>	1
<i>Rickettsia</i> , <i>Borrelia</i> , <i>Ehlichia</i> , <i>Anaplasma</i>	1
<i>Salmonella</i>	1
<i>Salmonella enteritidis</i>	1
<i>Salmonella sp.</i> , <i>Shigella sp.</i> , <i>Vibrio sp.</i>	1
<i>Salmonella typhimurium</i>	1
<i>Salmonella</i> , <i>Campylobacter</i> , EHEC	1
<i>Yersina pestis</i>	1

Table 3-4: Summary Excerpt of Relevant Articles on Zoonotic and/or Vector-Borne Parasites Associated with Military Missions by PubMed Research (Status of Search: 22 January 2013).

Parasite	Number of Found Relevant Articles
<i>Plasmodium sp.</i>	11
<i>Leishmania sp.</i>	8
<i>Plasmodium falciparum</i>	5
<i>Schistosoma mansoni</i>	5
<i>Blastocystis hominis</i>	4
<i>Leishmania tropica</i>	4
<i>Plasmodium vivax</i>	3
<i>Strongyloides stercoralis</i>	3
<i>Clonorchis sinensis</i>	2
<i>Giardia lamblia</i>	2
<i>Leishmania major</i>	2
<i>Metagonimus sp.</i>	2
<i>Schistosoma haematobium</i>	2
<i>Ascaris lumbricoides</i>	1

Parasite	Number of Found Relevant Articles
<i>Blastocystis sp.</i>	1
<i>Cryptosporidium parvum</i>	1
<i>Cryptosporidium sp.</i>	1
<i>Cyclospora sp.</i>	1
<i>Echinostoma hortense</i>	1
<i>Giardia intestinalis</i>	1
<i>Leishmania amazonensis</i>	1
<i>Leishmania brasiliensis ssp. Panamanensis</i>	1
<i>Leishmania braziliensis</i>	1
<i>Leishmania infantum</i>	1
<i>Leishmania lindenbergi</i>	1
<i>Leishmania naiffi</i>	1
<i>Neodiplostomum seoulensis</i>	1
<i>Oestrus ovis</i>	1
<i>Opisthorchis viverrini</i>	1
<i>Plasmodium knowlesi</i>	1
<i>Plasmodium sp; (mostly falciparum)</i>	1
<i>Plasmodium sp; (mostly vivax)</i>	1
<i>Toxoplasma gondii</i>	1

3.2.4 Discussion

The literature search was performed using PubMed. This methodology limits the search to published information and as such does not provide information on outbreaks or pathogen occurrences that are unpublished. There is no central NATO database or network that contains surveillance information documenting outbreaks or occurrences to inform our work.

3.3 PRIORITY LIST

3.3.1 Methodology

3.3.1.1 Priority Criteria: Definition

Other working groups have prioritized pathogens. The importance of clear criteria definitions used to prioritize pathogens is stressed in most of the reports created by these groups, in order to create a transparent and reproducible result.

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Within Belgian Defence, the opinion of a multidisciplinary team was gathered before the initial meeting in Neuilly-sur-Seine in Spring 2012.

Experts from the following disciplines and services were involved in the priority criteria definition and scoring:

- Physicians and veterinarians with operational experience from the Medical Operational Command.
- Occupational health physicians from the department of Well-Being.
- Infectiologists from the Queen Astrid Military Hospital Travel Clinic.
- Scientists from the Laboratory for Vector-borne Diseases.
- Transfusion medicine specialists from the Military Service for Blood Transfusion.
- Veterinarians from the Queen Astrid Military Hospital veterinary laboratory.

The main sources of information that were used were Mandell's Principles and Practice of Infectious Diseases and the article on Wildtool by Tavernier *et al.* [91].

The initial criteria were discussed in the aforementioned first meeting in Paris. A few additional criteria were added during the discussion.

'Natural risk in own country' and 'natural infection risk during deployments' were removed from the criteria during the 4th meeting in Ghent (Spring 2015) as it became clear that these criteria are not about the pathogen itself, and differ so greatly between the different member states that it makes less sense in this context to use for scoring. Later in this chapter we will discuss how the priority lists can be used.

3.3.1.2 Priority Criteria: Scoring Agreement

The initial scoring system was set up by the Belgian multidisciplinary team.

The scoring system was further defined during the RTG-meetings.

Table 3-5: Final Priority Criteria Scoring Agreement.

	Low	Average	High	Very High
Risk of biological warfare use	0	1	2	3
Emergent / growing threat	0	1	3	5
Psychological and political impact	0	1	3	5
Airborne transmission	0	1	3	5
Food / waterborne transmission	0	1	3	5
Bloodborne transmission	0	1	3	5
Difficulty of prevention	0	1	2	3
Difficulty of vaccination	0	1	2	3
Difficulty of environmental detection	0	1	2	3

	Low	Average	High	Very High
Difficulty of animal and human diagnosis	0	1	2	3
Difficulty of treatment	0	1	3	5
Severity of disease	0	2	6	10
Mortality risk	0	2	6	10

3.3.2 Scoring

Every member of RTG HFM-230 took the list of pathogens and the priority criteria back to specialists in their own country for scoring. Six countries were able to submit their scores. The results were added to an Excel table and mean values were calculated.

For pathogens that were added later (following the literature search), a consensus scoring was completed during the meeting in Rome in 2014. This is the case for alkurma virus, sindbis virus, o'nyong-nyong virus, nipah virus, hendra virus, *Staphylococcus aureus* and relapsing fever *Borrelia*.

The ranking was done based on the mean values. Minimum and maximum scores and standard deviations were calculated.

The scoring results were discussed during the 4th meeting in Rome and again during the 6th meeting in Ghent. All of the team members present in these meetings reviewed the final results for plausibility. The final ranking provoked a lot of discussion, leading to the recommendation to review the criteria and the weighting scores.

3.3.3 Result

3.3.3.1 Priority List: Viruses

Table 3-6: Ranking of the 30 Viruses.

Rank	Virus	Average Score (/75)	Minimum Score	Maximum Score	Standard Deviation
1	Marburg virus	53,00	45	59	4,56
2	Ebola virus	52,50	45	56	3,83
3	Lassa fever	45,83	32	60	9,22
4	Corona virus (SARS)	45,17	32	53	7,22
5	Crimean-Congo Hemorrhagic Fever virus	42,83	34	55	6,94
6	Rabies virus	41,83	29	48	7,63
7	Influenza viruses (zoonotic)	41,33	34	45	4,50
8	Nipah virus	41,00	<i>Consensus Scoring</i>		
9	Hendra virus	39,00	<i>Consensus Scoring</i>		

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Rank	Virus	Average Score (/75)	Minimum Score	Maximum Score	Standard Deviation
10	Rift Valley Fever virus	36,67	20	44	10,17
11	Kyanasur Forest Disease virus	31,17	20	39	8,80
12	Omsk Hemorrhagic Fever virus	31,00	21	35	6,03
13	Argentine Hemorrhagic Fever virus	30,67	22	40	6,77
14	Bolivian Hemorrhagic Fever virus	30,67	22	38	6,28
15	Pox viruses (zoonotic)	30,50	16	49	11,10
16	Hantaviruses	30,50	21	40	6,19
17	Herpes B virus	30,00	20	36	7,90
18	Yellow Fever virus	29,17	19	43	8,16
19	Alkhurma virus	29,00	<i>Consensus Scoring</i>		
20	Equine Encephalitis viruses	28,83	22	33	5,00
21	Dengue Fever virus	28,17	21	36	5,78
22	West Nile virus	27,00	20	33	6,00
23	Chikungunya virus	26,50	17	31	6,63
24	Hepatitis E virus	25,50	22	28	2,26
25	Lymphocytic Choriomeningitis virus	24,50	17	29	4,23
26	Japanese Encephalitis virus	24,33	19	28	3,50
27	Tickborne Encephalitis virus	21,83	15	25	4,58
28	Sandfly Fever viruses	20,50	16	28	4,23
29	Sindbis virus	17,00	<i>Consensus Scoring</i>		
30	O'Nyong-Nyong virus	17,00	<i>Consensus Scoring</i>		

3.3.3.2 Priority List: Bacteria

Table 3-7: Ranking of the 27 Bacteria.

Rank	Bacteria	Average Score (/75)	Minimum Score	Maximum Score	Standard Deviation
1	<i>Clostridium botulinum</i>	43,83	37	50	4,12
2	<i>Yersinia pestis</i>	43,00	39	51	4,38
3	<i>Bacillus anthracis</i>	42,83	33	52	6,01
4	<i>Coxiella burnettii</i>	37,00	30	47	5,62

Rank	Bacteria	Average Score (/75)	Minimum Score	Maximum Score	Standard Deviation
5	<i>Francisella tularensis</i>	35,50	32	39	3,21
6	<i>Burkholderia mallei/pseudomallei</i>	31,33	29	33	1,37
7	<i>Brucellae</i>	31,17	25	39	4,58
8	<i>Escherichia coli</i> enteropathogenic	30,50	22	38	6,16
9	<i>Rickettsiae</i>	29,83	22	35	6,37
10	<i>Mycobacterium bovis</i>	26,17	23	30	2,40
11	<i>Salmonellae</i>	24,67	23	30	2,73
12	<i>Leptospira interrogans</i>	21,83	18	29	4,36
13	<i>Yersinia pseudotuberculosis</i>	21,67	15	25	4,80
14	<i>Campylobacter</i>	21,00	16	24	3,95
15	<i>Streptococcus suis</i>	20,67	14	24	4,80
16	<i>Chlamydophila psittaci</i>	20,67	17	24	2,50
17	<i>Staphylococcus aureus</i>	20,00	Consensus Scoring		
18	<i>Listeria monocytogenes</i>	19,83	18	25	2,99
19	<i>Yersinia enterocolitica</i>	18,00	14	33	7,43
20	<i>Borrelia burgdorferi</i> sensu lato	17,67	12	28	5,43
21	<i>Ehrlichiae</i>	14,83	12	20	3,31
22	<i>Bartonellae</i>	14,00	12	17	2,00
23	<i>Anaplasma phagocytophilum</i>	13,67	10	20	3,33
24	<i>Streptobacillus moniliformis</i>	12,00	10	15	2,37
25	<i>Relapsing fever Borrelia</i>	12,00	Consensus Scoring		
26	<i>Pasteurella multocida</i>	11,83	10	15	1,94
27	<i>Spirillum minor</i>	10,83	7	15	3,37

3.3.3.3 Priority List: Parasites

Table 3-8: Ranking of the 24 Parasites.

Rank	Parasite	Average Score (/75)	Minimum Score	Maximum Score	Standard Deviation
1	<i>Echinococci</i>	37,67	27	47	6,53
2	<i>Plasmodiae</i>	31,50	24	43	8,04

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Rank	Parasite	Average Score (/75)	Minimum Score	Maximum Score	Standard Deviation
3	<i>Trypanosoma brucei</i>	26,50	24	32	3,21
4	<i>Leishmaniae</i>	26,00	19	31	4,52
5	<i>Trypanosoma cruzi</i>	25,67	19	42	8,26
6	<i>Trichinellae</i>	23,67	22	39	8,45
7	<i>Schistosomiae</i>	23,33	20	35	6,06
8	<i>Toxoplasma gondii</i>	20,50	14	39	10,01
9	<i>Giardia intestinalis</i>	20,17	17	27	4,92
10	<i>Angiostrongylus cantonensis</i>	20,00	13	29	7,07
11	<i>Wuchereria bancrofti</i>	19,67	13	32	7,94
12	<i>Cryptosporidium</i>	19,50	18	23	1,76
13	<i>Balantidium coli</i>	17,83	15	19	1,47
14	<i>Babesiae</i>	17,67	13	28	6,06
15	<i>Taeniae</i>	17,67	14	19	1,97
16	<i>Toxocarae</i>	15,50	11	24	5,43
17	<i>Strongyloides spp.</i>	15,50	10	20	4,12
18	<i>Fasciola hepatica</i>	15,17	14	18	1,60
19	<i>Chlonorchis sinensis</i>	14,17	13	18	2,04
20	<i>Ectoparasites</i>	13,83	10	16	2,32
21	<i>Ascarididae</i>	13,75	11	18	3,10
22	<i>Diphyllobotrium latum</i>	12,17	10	16	2,71
23	<i>Paragonimus westermani</i>	11,50	10	16	2,51
24	<i>Onchocerca volvulus</i>	11,00	9	16	2,90

3.3.3.4 Priority List: Overall

Table 3-9: Overall Ranking of All 81 Pathogens.

Overall Rank	Pathogen	Average Score (/75)	Minimum Score	Maximum Score	V/B/P
1	Marburg virus	53,00	45	59	V
2	Ebola virus	52,50	45	56	V

Overall Rank	Pathogen	Average Score (/75)	Minimum Score	Maximum Score	V/B/P
3	Lassa fever	45,83	32	60	V
4	Corona virus (SARS)	45,17	32	53	V
5	<i>Clostridium botulinum</i>	43,83	37	50	B
6	<i>Yersinia pestis</i>	43,00	39	51	B
7	Crimean-Congo Hemorrhagic Fever virus	42,83	34	55	V
8	<i>Bacillus anthracis</i>	42,83	33	52	B
9	Rabies virus	41,83	29	48	V
10	Influenza viruses (zoonotic)	41,33	34	45	V
11	Nipah virus	41,00	Consensus Scoring		V
12	Hendra virus	39,00	Consensus Scoring		V
13	<i>Echinococci</i>	37,67	27	47	P
14	<i>Coxiella burnettii</i>	37,00	30	47	B
15	Rift Valley Fever virus	36,67	20	44	V
16	<i>Francisella tularensis</i>	35,50	32	39	B
17	<i>Plasmodiae</i>	31,50	24	43	P
18	<i>Burkholderia mallei/pseudomallei</i>	31,33	29	33	B
19	Kyasanur Forest Disease virus	31,17	20	39	V
20	<i>Brucellae</i>	31,17	25	39	B
21	Omsk Hemorrhagic Fever virus	31,00	21	35	V
22	Argentine Hemorrhagic Fever virus	30,67	22	40	V
23	Bolivian Hemorrhagic Fever virus	30,67	22	38	V
24	Pox viruses (zoonotic)	30,50	16	49	V
25	Hantaviruses	30,50	21	40	V
26	<i>Escherichia coli</i> enteropathogenic	30,50	22	38	B
27	Herpes B virus	30,00	20	36	V
28	<i>Rickettsiae</i>	29,83	22	35	B
29	Yellow Fever virus	29,17	19	43	V
30	Alkhurma virus	29,00	Consensus Scoring		V
31	Equine Encephalitis viruses	28,83	22	33	V

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Overall Rank	Pathogen	Average Score (/75)	Minimum Score	Maximum Score	V/B/P
32	Dengue Fever virus	28,17	21	36	V
33	West Nile virus	27,00	20	33	V
34	Chikungunya virus	26,50	17	31	V
35	<i>Trypanosoma brucei</i>	26,50	24	32	P
36	<i>Mycobacterium bovis</i>	26,17	23	30	B
37	<i>Leishmaniae</i>	26,00	19	31	P
38	<i>Trypanosoma cruzi</i>	25,67	19	42	P
39	Hepatitis E virus	25,50	22	28	V
40	<i>Salmonellae</i>	24,67	23	30	B
41	Lymphocytic Choriomeningitis virus	24,50	17	29	V
42	Japanese Encephalitis virus	24,33	19	28	V
43	<i>Trichinellae</i>	23,67	22	39	P
44	<i>Schistosomiae</i>	23,33	20	35	P
45	Tickborne Encephalitis virus	21,83	15	25	V
46	<i>Leptospira interrogans</i>	21,83	18	29	B
47	<i>Yersinia pseudotuberculosis</i>	21,67	15	25	B
48	<i>Campylobacter</i>	21,00	16	24	B
49	<i>Streptococcus suis</i>	20,67	14	24	B
50	<i>Chlamydomydia psittaci</i>	20,67	17	24	B
51	Sandfly Fever viruses	20,50	16	28	V
52	<i>Toxoplasma gondii</i>	20,50	14	39	P
53	<i>Giardia intestinalis</i>	20,17	17	27	P
54	<i>Staphylococcus aureus</i>	20,00	Consensus Scoring		B
55	<i>Angiostrongylus cantonensis</i>	20,00	13	29	P
56	<i>Listeria monocytogenes</i>	19,83	18	25	B
57	<i>Wuchereria bancrofti</i>	19,67	13	32	P
58	<i>Cryptosporidium</i>	19,50	18	23	P
59	<i>Yersinia enterocolitica</i>	18,00	14	33	B
60	<i>Balantidium coli</i>	17,83	15	19	P

Overall Rank	Pathogen	Average Score (/75)	Minimum Score	Maximum Score	V/B/P
61	<i>Borrelia burgdorferi</i> sensu lato	17,67	12	28	B
62	<i>Babesia</i>	17,67	13	28	P
63	<i>Taenia</i>	17,67	14	19	P
64	Sindbis virus	17,00	Consensus Scoring		V
65	O'Nyong-Nyong virus	17,00	Consensus Scoring		V
66	<i>Toxocara</i>	15,50	11	24	P
67	<i>Strongyloides</i> spp.	15,50	10	20	P
68	<i>Fasciola hepatica</i>	15,17	14	18	P
69	<i>Erlichia</i>	14,83	12	20	B
70	<i>Chlonorchis sinensis</i>	14,17	13	18	P
71	<i>Bartonella</i>	14,00	12	17	B
72	<i>Ectoparasites</i>	13,83	10	16	P
73	<i>Ascarididae</i>	13,75	11	18	P
74	<i>Anaplasma phagocytophilum</i>	13,67	10	20	B
75	<i>Diphyllobotrium latum</i>	12,17	10	16	P
76	<i>Streptobacillus moniliformis</i>	12,00	10	15	B
77	Relapsing fever <i>Borrelia</i>	12,00	Consensus Scoring		B
78	<i>Pasteurella multocida</i>	11,83	10	15	B
79	<i>Paragonimus westermani</i>	11,50	10	16	P
80	<i>Onchocerca volvulus</i>	11,00	9	16	P
81	<i>Spirillum minor</i>	10,83	7	15	B

3.3.4 Discussion

The RTG HFM-230 was established with the understanding of the importance of a concerted action towards detection and surveillance of zoonotic and vector-borne disease with dissemination of this information between NATO partners. The RTG HFM-230 undertook the difficult task of attempting to prioritize the selected pathogens and provide diagnostic and identification methods for each of them as outlined in the Technical Activity Proposal (TAP).

As Krause *et al.* [43] describe in their paper “How can infectious diseases be prioritized in public health?” prioritizing pathogens is inherently challenging. Factors contributing to this difficulty include deficiencies in knowledge of the pathogen, defining criteria by which to judge pathogen importance, and understanding criteria may be affected by medical, cultural or political factors. In addition, determining appropriate weighting schemes

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for each of the criteria presents further challenges. Established criteria may have significant impact on priority ranking outcomes. For example, utilization of the criteria “food or waterborne transmission” placed additional emphasis on certain pathogens while decreasing emphasis on other pathogens. As recent infectious disease outbreaks have demonstrated, the scientific and medical communities have significant knowledge gaps for many infectious diseases. These knowledge gaps adversely affect the ability to establish criteria and weighting scores potentially resulting in over or underestimation for certain pathogens [91].

The criteria utilized for determining priority ranking were modified during the meetings based on extensive discussions and finally focused on properties of the pathogens. For example the criterion “risk of infection during deployment” was removed because of the significant variation in deployment locations, and therefore variation in disease exposures, for the member countries. Similarly, the criterion “natural risk of infection in own country” was removed. Deletion of these two criteria helped to diminish some country location and mission specific bias. Despite these adjustments to the criteria, there remains substantial variation between group member states. A further change to the criteria involved separating the criteria “severity of disease” and “mortality risk” which previously had been combined into one criterion. While some may have combined the criteria for prevention and vaccination, it was felt that vaccination is a unique method of prevention and varies in effectiveness and as such warranted a separate criteria category.

Some pathogens selected for prioritization were combined into one group. For example the poxviruses were combined into one group and the ectoparasites were also combined into one group. Because of the variation in pathological significance, it was difficult for them to be scored as a group. While separating them would add to the length of the list, future efforts may find it advantageous to separate the individual pathogens out.

Of interest is the wide range of standard deviations between individual country rankings. This may be a reflection of the differing impact of the various pathogens on the individual countries. Other possible explanations include pathogen knowledge gaps and unclear definitions of the criteria and weighting scores. Three pathogens had standard deviations of greater than ten:

- 1) *Toxoplasma*;
- 2) Rift Valley Fever virus; and
- 3) The poxviruses.

Further open discussion to share the rationale behind individual country rankings, experiences and perspectives may decrease the standard deviations.

The priority list is a tool that must be reviewed in the context in which it was developed. It is not an “all in one device suitable for every purpose”. While it may not reflect the priority for an individual country, it provides a starting point for discussion and possible decisions concerning pathogen research and medical countermeasure development.

This work should be viewed as a living document with full expectation that the priority ranking will change over time as new pathogens emerge, the knowledge base for a pathogen increases, and medical diagnostics and countermeasures are developed. The priority ranking will also change as future group members change, both as relates to members’ NATO country of origin as well as professional background. It is this group’s recommendation that the future group include more NATO country representation. Additionally, it is recommended that future groups expand the multidisciplinary nature of the team to include e.g., veterinarians, microbiologists, virologists, epidemiologists, infectious disease physicians and clinical (to include mobile) laboratory specialists. It may be beneficial to provide a guide outlining suggested specialties to provide input to

individual country priority lists and ensuring criteria definitions and weighting scores are utilized consistently. It is also advisable that this priority ranking be redone on a regular basis (every 2 – 3 years) as new and emerging pathogens are recognized, medical countermeasures are developed and detection methods improve.



Chapter 4 – BIOSAFETY LEVELS

4.1 INTRODUCTION

A biosafety level is the level of the biocontainment precautions required for activities involving infectious microorganisms and laboratory animals. It is therefore a form of protection for the working staff and the environment against the pathogen.

The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4).

In the United States, the Centers for Disease Control and Prevention (CDC) have specified these levels (http://www.cdc.gov/biosafety/publications/bmbl5/BMML5_sect_IV.pdf).

In the European Union, the same biosafety levels are defined in a directive. (DIRECTIVE 2000/54/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work; seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC).

In the following subchapter the lab properties, practices and safety equipment of the four BSL levels are summarized (text in most parts from http://www.cdc.gov/biosafety/publications/bmbl5/BMML5_sect_IV.pdf).

Table 4-1: Summary Table of the Biosafety Levels.

Risk Group	Biosafety Level	Laboratory Type	Laboratory Practices	Safety Equipment
1	Basic – BSL-1	Basic teaching, research	GMT	None; open bench work
2	Basic – BSL-2	Diagnostic services, research	GMT plus protective clothing, biohazards sign	Open bench plus BSC for aerosols
3	Containment BSL-3	Special diagnostic services, research	Level 2 + special clothing, access control, directed airflow	BSC and/or other primary devices for all activities
4	Maximum Containment BSL-4	Dangerous pathogen units	Level 3 + airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suites with class II BSCs, double ended autoclave

4.2 BIOSAFETY LEVEL 1 (BSL-1)

BSL-1 is suitable for work involving well-characterized agents that are not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used but may be used as determined by appropriate risk assessment (Class I biosafety cabinet, BSC). Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

4.3 BIOSAFETY LEVEL 2 (BSL-2)

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

- 1) Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;
- 2) Access to the laboratory is restricted when work is being conducted; and
- 3) All procedures in which infectious aerosols or splashes may be created are conducted in BSCs Class II or other physical containment equipment.

4.4 BIOSAFETY LEVEL 3 (BSL-3)

BSL-3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices.

A BSL-3 laboratory has special engineering and design features. A BSL-3 laboratory is located away from common access.

4.5 BIOSAFETY LEVEL 4 (BSL-4)

BSL-4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

There are two models for BSL-4 laboratories:

- a) Cabinet Laboratory – Manipulation of agents must be performed in a Class III BSC; and
- b) Suit Laboratory – Personnel must wear a positive pressure supplied air protective suit.

BSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

The Biosafety Level 4 facility itself is often a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.



Chapter 5 – SAMPLING AND TRANSPORTATION OF SAMPLES

5.1 INTRODUCTION

The importance of appropriate specimen sampling to include accurate documentation, storage and transportation for either field or stationary labs cannot be under emphasized. The consequences of inappropriate sampling may include false negative or positive results, untraceable samples, compromised samples, and inadequate sample size. Utilization of technical experts from within NATO will inform and enhance both medical and nonmedical military capabilities.

5.2 THINGS TO REMEMBER AHEAD OF SAMPLING

In order to optimize sampling, RTG HFM-230 recommends the following:

- Check the logistic possibilities / constraints in the area of sampling, e.g.,:
 - What kind of temperature is normal in the area of operation (e.g., cold, temperate, tropical) humidity (arid, semiarid, humid, wet)?
 - What kind of storage and transport capabilities and capacities are available (chilled, frozen, room temperature)?
 - Method of transport (e.g., car, rotatory wing, fixed wing, ship).
 - How does the security in the area of operation influence sampling and diagnostics (safe / unsafe, stable / unstable)?
 - All restrictions and requirements must be in line with the Commander's guidance thereby enabling planned sampling and testing to be in accordance with the overall mission strategy.
- Depending on the actual ecology on site, a sufficient epidemiological sampling strategy including epidemiological study design is needed to estimate prevalences as accurate as possible. Include knowledge of specialists (e.g. epidemiology, microbiology, entomology).
- Delineate initial interdisciplinary screening investigations prior to or at the beginning of the mission form continuous monitoring during the mission, based on the initial findings.
- Initial findings define the testing needed for continuous monitoring and the lab capability.
- Use well defined and characterized tests to answer the question being asked (e.g., serology vs. pathogen detection; field vs. lab diagnostics).
- Identify testing required in the field as opposed to that which can or should be done at a fixed facility.
- Define the type of samples needed (swabs, blood, serum, feces, tissue, etc.).
- Based on expected pathogens present, identify needed fixation methods (e.g., formic acid, lysis buffer, 70% alcohol, etc.) ahead of diagnostics or transport.
- The type of sample and the infectious diseases present in the area of sampling define the classification for transport according to UN-regulations. The UN-classification results in regulations for storage and transport.

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- Infectious substances are substances which are known or are reasonably expected to contain pathogens. Pathogens are defined as micro-organisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans or animals. Infectious substances are classified in Division 6.2 and assigned to UN 2814, UN 2900, UN 3291 or UN 3373, as appropriate (WHO/HSE/GCR/2015.2: Guidance on regulations for the transport of infectious substances 2015 – 2016):
 - Follow the Packaging Instructions P620 for Infectious Substances in Category A (UN 2814, UN 2900) or the United Nations packaging Instruction P650 for transport of Infectious Substances in Category B (UN 3373). For medical or clinical wastes containing infectious substances in Category B, or which are reasonably believed to have a low probability of containing infectious substances, shall be assigned to UN 3291 and shipped following Packing Instruction P621 (ICAO/IATA PI622).
- Clarify export regulations of the country where the mission takes place and import regulations into the country of expert lab, concerning (selection):
 - IATA-regulations (mode of transport, declaration).
 - Infectious diseases (veterinary point of view).
 - Infectious diseases (medical point of view).
 - EU-regulations (for EU member states).
 - Dual use agents.
 - Wildlife conservation.
 - Hazardous substances (dried ice, formic acid, etc.).
 - GMO.

Table 5-1: Summary Table of UN Classifications for Shipping of Samples.

Category	Classification	Description	Proper Shipping Name
A	UN 2814	An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals (zoonotic).	INFECTIOUS SUBSTANCE; INFECTING HUMANS
	UN 2900	An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy animals (not zoonotic).	INFECTIOUS SUBSTANCE; INFECTING ANIMALS
B	UN 3373	An infectious substance which does not meet the criteria for inclusion in Category A. Infectious substances in Category B must be assigned to UN 3373.	BIOLOGICAL SUBSTANCE CATEGORY B

Category	Classification	Description	Proper Shipping Name
B	UN 3291	Medical or clinical wastes containing infectious substances in Category B, or which are reasonably believed to have a low probability of containing infectious substances, shall be assigned to UN 3291. NOTE: Medical or clinical wastes containing Category A infectious substances shall be assigned to UN 2814 or UN 2900 as appropriate.	“CLINICAL WASTE, UNSPECIFIED, N.O.S.” or “(BIO) MEDICAL WASTE, N.O.S.” or “REGULATED MEDICAL WASTE, N.O.S.”.

5.3 HELPFUL LINKS CONCERNING REGULATIONS AND TRANSPORT

- <http://www.unece.org/trans/danger/danger.html>
 UN Recommendations on the Transport of Dangerous Goods – homepage.
- http://apps.who.int/iris/bitstream/10665/149288/1/WHO_HSE_GCR_2015.2_eng.pdf
 WHO/HSE/GCR-guidance on regulations for the Transport of Infectious Substances 2015 – 2016.
- <http://www.enivd.de/psreg.htm>
 Listing of links to European, U.S. and international organizations for packaging and shipping Regulations.
- http://www.unece.org/trans/danger/publi/adr/adr2013_amend.html
 European Agreement concerning the International Carriage of Dangerous Goods by Road Amendments for entry into force on 1 January 2015.
- <http://www.icao.int/safety/DangerousGoods/Pages/technical-instructions.aspx>
 Technical Instructions for the Safe Transport of Dangerous Goods by Air (Doc 9284).
- <http://www.iata.org/whatwedo/cargo/dgr/Pages/download.aspx>
 IATA infectious substances transport (Transport of Dangerous Goods by Air).
- <http://www.imo.org/en/Pages/Default.aspx>
 Safe Transport of Dangerous Goods by ship.
- <http://phmsa.dot.gov/hazmat/transporting-infectious-substances>
 U.S. Department of Transportation.
- <http://www.cdc.gov/dpdx/diagnosticProcedures/index.html>
 Examples for labeling, documentation, fixation, shipping (e.g., whole blood).

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- http://trade.ec.europa.eu/doclib/docs/2008/september/tradoc_140595.pdf
List of Dual Use items and Technology.
- <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:134:0001:0269:de:PDF>
VO (EU) 428/2009 concerning the import, transport of Dual Use items and Technology.
- http://www.wfcc.info/pdf/wfcc_transport.pdf
World Federation of Culture Collections (WFCC) Information Resource on International Postal Regulations for Shipping Biological Materials The current UPU regulations and their background.
- https://www.dsmz.de/fileadmin/Bereiche/Microbiology/Dateien/EBRCN_information_transport_August_2013.pdf
European Biological Resource Centers – Network Information Resource (EBRCN): International Regulations for Packaging and Shipping of Microorganisms.

5.4 THE UN-CATEGORIZATION DEFINES THE WAY OF PACKAGING FOR TRANSPORT / SHIPPING

5.4.1 UN 3373

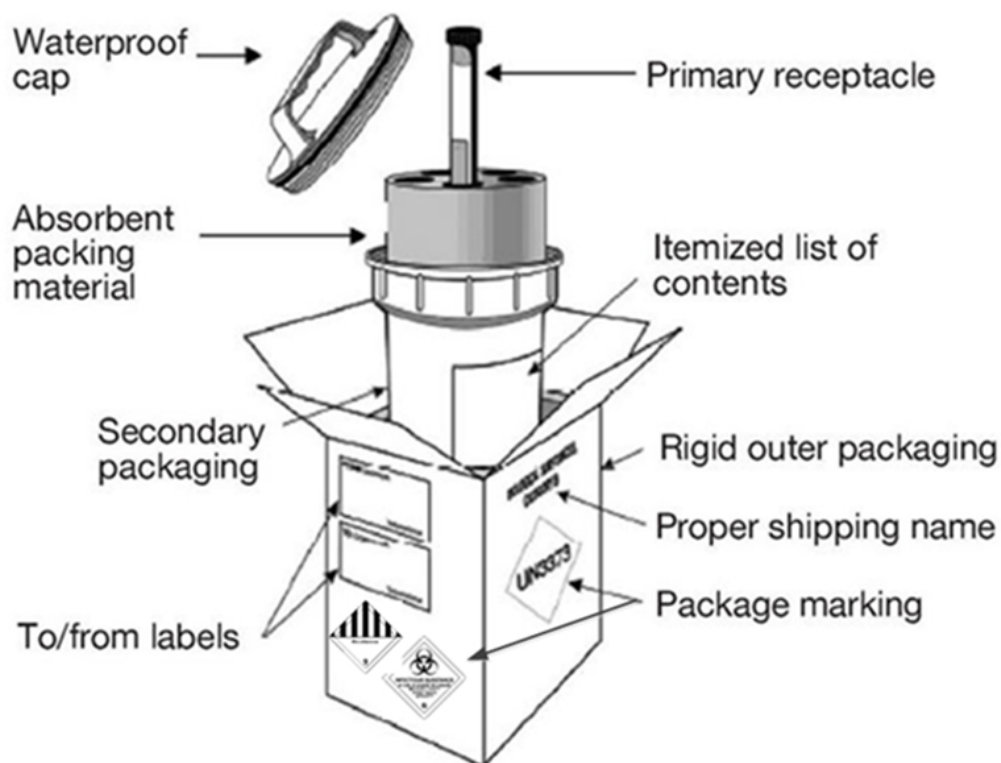


Figure 5-1: UN 3373 Shipping Packaging.

5.4.2 UN 2814

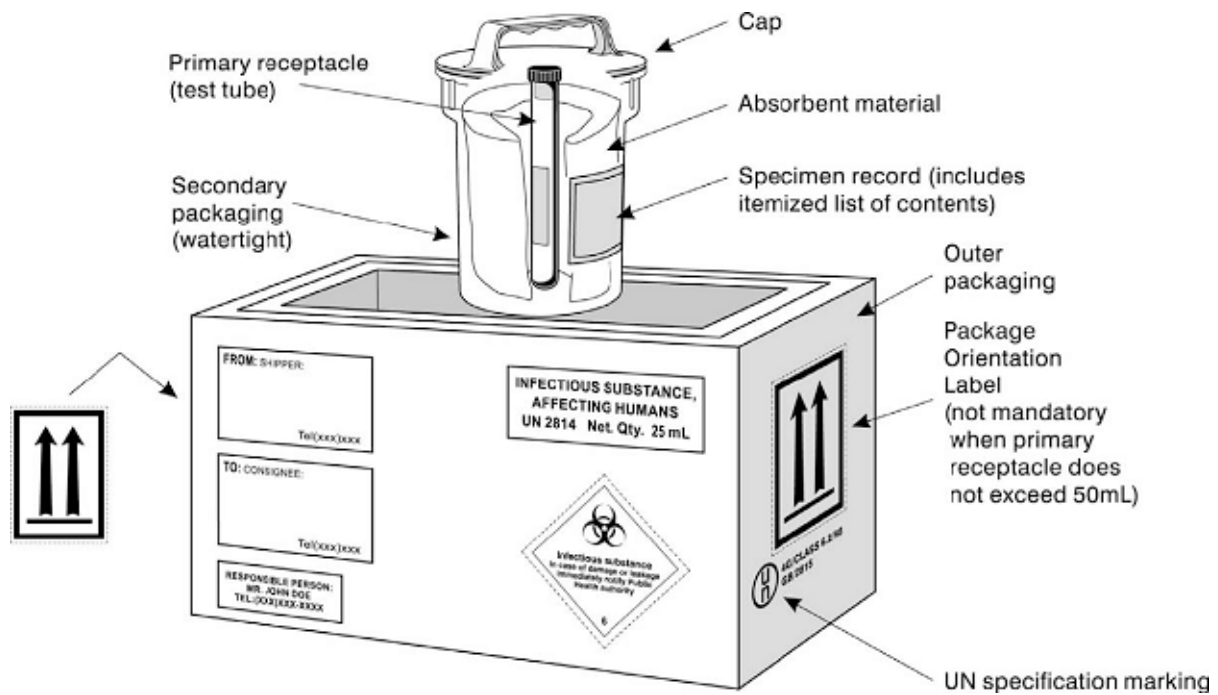


Figure 5-2: UN 2814 Shipping Packaging.



Chapter 6 – DETECTION METHODS

6.1 INTRODUCTION

Sampling and detection of pathogens is a rapidly growing field. Many techniques and technologies are still under development. There are various factors to consider when sampling for pathogens, such as method of dispersion of the pathogen, purpose of the sampling (e.g., to identify the pathogen, determine extent of contamination, confirm decontamination, etc.), environmental conditions, persistence of the pathogen, physical state of the pathogen, area/volume to be sampled, laboratory protocols, and others. It is important to note that biological pathogens are particulate matter, and, therefore, detection methods are designed for particulate sampling.

In case of an outbreak, we expect not only human clinical samples like blood, sputum, urine, stool, CSF, etc., but also food and water samples and environmental samples. Appropriate sample preparation is needed in most of the systems that have been developed with either the nucleic acid or antibody based detection systems. Sample preparation takes hours to days depending on the standardized protocols and often cannot be performed in field conditions. Depending on sampling techniques and transport methods, samples of bacteria from the field may not be viable for culture. The sample collection procedures, handling, transportation and preparation are vital for correct identification. Although the sample preparation and efficacy of extraction procedures determine the concentration of the pathogen availability for detection, in some instances, the viability of virus or bacteria has to be confirmed by conventional culture methods. Conventional detection and identification of human pathogens usually relies on culture and biochemical tests and biochemical tests that take minimum 3 – 7 days and in some cases even more than 15 days to provide results. The culture and biochemical tests are reliable but need highly trained manpower to be performed. Additionally they do not provide results on a real time basis and as such cannot be used in situations that need immediate results. Efforts are currently on to develop methods that are rapid, accurate and can detect the pathogens in the field as well as in laboratories that have limited or no instrumental support.

The most commonly used laboratory methods for the detection of viruses and virus components in biological samples can be broadly divided into 3 categories – those that measure virus infectivity, those that examine viral serology (that is, host antibody responses to the virus), and those that rely on molecular methods. One method, which does not precisely fit into any of these categories, is the use of imaging techniques to directly visualize virus particles.

Detection methods included are simple Polymerase Chain Reaction (PCR), multiplex PCR, real-time PCR, Nucleic Acid Sequence-Based Amplification (NASBA), Loop-mediated isothermal Amplification (LAMP) and oligonucleotide DNA microarray which classified as nucleic acid-based methods; optical, electrochemical and mass-based biosensors which classified as biosensor-based methods; Enzyme-Linked Immunosorbent Assay (ELISA) and lateral flow immunoassay which classified as immunological-based methods. In general, rapid detection methods are generally time-efficient, sensitive, specific and labour-saving. The developments of rapid detection methods are vital in prevention and treatment of vector-borne and zoonotic diseases.

The chapter on the detection methods of viruses is based on methods that are available in classical diagnostic stationary and field laboratories. Isolation and cultivation of viruses are only established in special laboratories with unique requirements. Therefore these are not included in the list of detection methods for viruses.

The sensitive and specific detection of a broad spectrum of bacteria is possible using cultural techniques. These methods require laboratories with equipment as appropriate and need at least 24 hours for results. Fast and

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reliable detection methods are needed in the area of operation. The decision, which tests are sufficient for first line detection, focused on the ease of use as well as its robustness. In addition, for some bacteria the microscopy of stained smears gives very fast and specific results. Nevertheless, all diagnostic methods require the medical background to interpret the results in face of clinical symptoms, epidemiology and environmental circumstances.

For the majority of parasites, detection relies on microscopy. RTG-230 considers this method as a first-line detection method, applicable in a field setting with a minimum of material needed. However, microscopic detection requires specialized personnel for the accurate identification of the specimen. The ‘ease of use’ is thus not fully comparable with other first line detection methods such as hand held lateral flow assays, which can be handled with a minimum of training.

In our opinion a microscope and a thoroughly trained technician to perform the identification is indispensable in a field laboratory setting when parasitic pathogens might be encountered. A reliable source with good pictures for identification is helpful. For example, CDC’s Division of Parasitic Diseases and Malaria (DPDM) uses the Internet to assist laboratorians and pathologists in the diagnosis of parasitic diseases, both in the United States and abroad. More information and contact through <http://www.cdc.gov/dpdx/contact.html>. Comparative figures for parasites in stool samples can be found here: <http://www.cdc.gov/dpdx/diagnosticProcedures/stool/morphcomp.html>.

Where applicable, an internet link to pictures is provided in the detection method table per pathogen in this chapter.

6.2 DEFINITION OF FIRST AND SECOND LINE DETECTION METHODS

“Diagnostic in the field” comprises simple, easy to use and robust tests, which can be used without specific infrastructure. Generally they can be done in tents, containers or vehicles, which have a power source at a minimum. Indirect detection methods, such as lateral flow assays for antibodies, do not differentiate active infection from prior exposure or illness. The use of microscopic detection methods can supplement this approach especially if parasites and some bacteria are of significance. Although these diagnostic methods may lack specificity, when taken together with a clinical history and physical exam findings, they may provide useful information. Due to their lack of sensitivity they are not sufficient for the detection of disease in reservoirs or vectors and therefore not adequate for monitoring which enables specific recommendations for preventive measures.

“Diagnostics in the lab” enable a more advanced, sensitive and specific detection of pathogens due to the use of molecular, cultural or advanced serologically tests. Moreover, in this approach they facilitate adequate testing methods for a continuous monitoring of possible reservoirs and vectors. In this approach a specified laboratory infrastructure is needed, either constructed as a solid building or a mobile container-based solution. Additional testing, e.g. for molecular epidemiology are possible in cooperation with expert labs in the native country of the member states. The personnel needed in this case should be experienced laboratory specialists (e.g., microbiologist) with additional technical assistance.

6.3 METHOD CLASSIFICATION

In the following chapter RTG HFM-230 gives a comprehensive and detailed overview on detection methods. The lists contain methods that could be applicable in first line in the field where more or less equipment is available.

Despite the inevitable gaps the panel tried to make this overview as sophisticated as possible. Therefore several codes have been introduced in order to give further information on the listed detection methods (last column of tables in Sections 6.4, 6.5 and 6.6):

- For pathogens where the group found commercial assays, it was stated if the listed assay is by the company either recommended:
 - “For diagnostic use with verification, approved by FDA or CE-certified” signed with abbreviation “D” in code; or
 - “For research only” marked with abbreviation “R” in code; or
 - “In vitro diagnostic only” is designated “IVD” in the code; or
 - If there was no sufficient information supplied by the company the test was marked with “nk” (for not known).
- For some pathogens the group recommends in house assays taken from the literature. In this cases the papers are cited and the panel provided information as to if:
 - The assay was used and validated by RTG-230 panel member for diagnostic purpose. In this case the method is marked with “U”.
 - The assay has not been tested by a RTG-230 member. In this case the mentioned assays are coded with an “L”.
- For some detection methods such as parasite detection by microscopy further information was not applicable and in this case the code “N/A” was introduced.

Table 6-1: Summary of Detection Method Classification.

For research only	R
For diagnostic use with verification, approved by FDA or CE-certified	D
Used and validated by RTG HFM-230 member	U
Literature recommendation only, not tested by RTG HFM-230 member	L
Not applicable (for example for the parasite detection by microscopy)	N/A
Use for diagnostics unknown	nk
In vitro diagnostic only	IVD

6.4 DETECTION METHODS OF VIRUSES

6.4.1 Alkhurma Virus

Bio Safety Level 3.

DETECTION METHODS

6.4.1.1 PSDS

<http://www.cdc.gov/vhf/alkhurma/>.

http://ecdc.europa.eu/en/healthtopics/Alkhurma_haemorrhagic_fever/Pages/factsheet-health-professionals.aspx.

6.4.1.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.1.3 Second Line Detection ‘In the Lab’

Table 6-2: Second Line Detection Alkhurma Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, plasma		Alkhurma virus PCR TIB MolBio http://www.tib-molbiol.com/	L
rtRT-PCR	Serum, plasma		Carletti <i>et al.</i> [9]	L
rtRT-PCR	Serum, plasma			L
ELISA/IFA	Serum, EDTA-plasma	Exists in specialized laboratories		

6.4.2 Argentine Hemorrhagic Fever Virus (Junin Virus)

Bio Safety Level 4.

6.4.2.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/junin-eng.php>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=215>.

6.4.2.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.2.3 Second Line Detection ‘In the Lab’

Table 6-3: Second Line Detection Argentine Hemorrhagic Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor, urine, viscus		Vieth <i>et al.</i> [99]	U

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor, urine, viscus		Trombley <i>et al.</i> [94]	L
RT-PCR	Serum, EDTA-plasma, liquor, urine, viscus		Lozano <i>et al.</i> [56]	L
ELISA/IFA	Serum, EDTA-plasma	Exists in specialized laboratories		

6.4.3 Bolivian Hemorrhagic Fever Virus (Machupo Virus)

Bio Safety Level 4.

6.4.3.1 PSDS

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=274>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/machupo-eng.php>.

6.4.3.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.3.3 Second Line Detection ‘In the Lab’

Table 6-4: Second Line Detection Bolivian Hemorrhagic Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor, urine, viscus		Vieth <i>et al.</i> [99]	U
rtRT-PCR	Serum, EDTA-plasma, liquor, urine, viscus		Trombley <i>et al.</i> [94]	L
ELISA/IFA	Serum, EDTA-plasma	Exists in specialized laboratories		

6.4.4 Chikungunya Virus

Bio Safety Level 3.

6.4.4.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/chikungunya-eng.php>.

DETECTION METHODS

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=108>.

<http://www.cdc.gov/chikungunya/fact/index.html>.

6.4.4.2 First Line Detection ‘In the Field’

Table 6-5: First Line Detection Chikungunya Virus.

Test Method	Sample	Comment	Reference/Company	Code
LAMP	Serum		Parida <i>et al.</i> [72]; Reddy <i>et al.</i> [81]; Lu <i>et al.</i> [57]	L
Solid phase immunochromatographic assay IgM Ab	Serum, plasma, whole blood	There might be cross-reaction with other alphaviruses	SD BIOLINE Chikungunya IgM http://www.standardia.com/en/home/product/rapid/infectious-disease/Anti-Chikungunya_IgM.html	nk
Lateral flow chromatographic immunoassay IgM Ab	Serum, plasma, whole blood	There might be cross-reaction with other alphaviruses	Chikungunya IgM Combo Rapid Test http://ctkbiotech.com/ctk-product/chikungunya-igm-combo-rapid-test/	nk

6.4.4.3 Second Line Detection ‘In the Lab’

Table 6-6: Second Line Detection Chikungunya Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma	Pls. read kit instructions for samples and platforms	RealStar ® Chikungunya RT-PCR Kit 2.0 http://www.altona-diagnostics.com/realstar-product-list-EN.html	D
rtRT-PCR	Serum, EDTA-plasma		TibMolBiol Kit Chikungunya https://www.tib-molbiol.com	R
rtRT-PCR	Serum, EDTA-plasma	Sample material see kit instructions	Non-structural protein 2 (nsp2) Primerdesign tests genesig Standard Kit http://www.genesig.com/assets/files/chikv_std.pdf	R
rtRT-PCR	Serum, EDTA-plasma	Sample material see kit instructions	Chikungunya Virus Real Time RT-PCR Kit http://www.genwaybio.com	R

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma	Sample material see kit instructions	Chikungunya RG quantitative real-time PCR Kit (Geno-sens) https://www.labortechnik.com/en/geno-sens	D
rtRT-PCR	Serum, EDTA-plasma		Pastorino <i>et al.</i> [73]	U
RT-PCR	Serum, EDTA-plasma		Pfeffer <i>et al.</i> [75]	U
IFAT Ab IgM/IgG	Serum	There might be cross-reaction with other alphaviruses	Euroimmun Chikungunya virus IFAT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA Ab IgM/IgG	Serum	There might be cross-reaction with other alphaviruses	Euroimmun Anti-Chikungunya Virus ELISA http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA Ab IgM/IgG	Serum, plasma		IBL Chikungunya IgM μ -capture/IgG ELISA http://www.ibl-international.com	R
ELISA Ab IgM/IgG	Serum, plasma	There might be cross-reaction with other alphaviruses	Abcam Anti-CHIKV IgG/IgM Human ELISA kit http://www.abcam.com/chikungunya-virus-igm-human-elisa-kit-ab177848.html	R
ELISA Ab IgM/IgG	Serum, plasma	There might be cross-reaction with other alphaviruses	NovaLisa™ Chikungunya Virus IgG/IgM capture ELISA http://www.novatec-id.com/fileadmin/user_upload/Flyers/Chikungunya-engl-2013.pdf	R
ELISA Ab IgM/IgG	Serum, plasma	There might be cross-reaction with other alphaviruses	Chikungunya Virus IgM μ -capture/IgG capture ELISA, genwaybio http://www.genwaybio.com	R
ELISA Ab IgM/IgG	Serum	There might be cross-reaction with other alphaviruses	SD Chikungunya IgM ELISA http://www.standardia.com/en/home/product/ELISA/Anti-Chikungunya_IgM_ELISA.html	R

DETECTION METHODS

6.4.5 Coronavirus (SARS)

Bio Safety Level 3.

6.4.5.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/sars-sras-eng.php>.

<http://www.cdc.gov/sars/about/fs-SARS.pdf>.

6.4.5.2 First Line Detection ‘In the Field’

Table 6-7: First Line Detection Coronavirus (SARS).

Test Method	Sample	Comment	Reference/Company	Code
LAMP	Nasopharyngeal aspirates		Poon <i>et al.</i> [79]	L

6.4.5.3 Second Line Detection ‘In the Lab’

Table 6-8: Second Line Detection Coronavirus (SARS).

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Sputum, serum, Bronchoalveolar Lavage (BAL), nasopharyngeal wash, swabs, lung tissue		Qiagen-artus SARS RG RT-PCR Kit RUO https://www.qiagen.com/ca/shop/detection-solutions/human-pathogens/artus-sars-rg-rt-pcr-kit-ruo/	R
rtRT-PCR	Sputum, serum, BAL, nasopharyngeal wash, swabs, lung tissue		CDC primers http://www.who.int/csr/sars/CD/Cprimers.pdf?ua=1	L
rtRT-PCR	Sputum, serum, BAL, nasopharyngeal wash, swabs, lung tissue		WHO PCR primers for SARS http://www.who.int/csr/sars/primers/en/	L
rtRT-PCR	Sputum, serum, BAL, nasopharyngeal wash, swabs, lung tissue		SARS RG quantitative real-time PCR kit (Geno-sens) https://www.labortechnik.com/en/geno-sens	D
rtRT-PCR	Sputum, serum, BAL, nasopharyngeal wash, swabs, lung tissue		Drosten <i>et al.</i> [21]	L
rtRT-PCR	Sputum, serum, BAL, nasopharyngeal wash, swabs, lung tissue	SARS, modification of Drosten <i>et al.</i> , [21] assay	Bressler & Nolte [8]	L

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Sputum, serum, BAL, nasopharyngeal wash, swabs, lung tissue		Poon <i>et al.</i> [78]	L
rtRT-PCR	Sputum, serum, BAL, nasopharyngeal wash, swabs, lung tissue		Adachi <i>et al.</i> [4]	L
IFAT	Serum		EUROIMMUN SARS-CoV IgG/IgM IIFT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA	Serum, plasma		PLATELIA SARS Screen (FRUO) www.biorad.com	R

6.4.6 Crimean-Congo Hemorrhagic Fever Virus

Bio Safety Level 4.

6.4.6.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/crim-congo-eng.php>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=122&SID=7>.

6.4.6.2 First Line Detection ‘In the Field’

Table 6-9: First Line Detection Crimean-Congo Hemorrhagic Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
LAMP	Serum		Osman <i>et al.</i> [67]	L

6.4.6.3 Second Line Detection ‘In the Lab’

Table 6-10: Second Line Detection Crimean-Congo Hemorrhagic Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum		Real Star®CCHFV RT-PCR Kit 1.0 http://www.altona-diagnostics.com/realstar-product-list-EN.html	R

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Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum		Congo Crimea Real Time PCR kit http://www.sacace.com	R
rtRT-PCR	Serum, EDTA-plasma	Might not detect all actual strains	Drosten <i>et al.</i> [20]	U
rtRT-PCR	Serum, EDTA-plasma	Strains change quickly, has eventually be adapted; detects all until 2015 described strains with high specificity and sensitivity	Atkinson <i>et al.</i> [5]	U
ELISA	Serum		CCHF IgG Sandwich ELISA Biological Diagnostic Supplies Limited, UK	R
IFAT	Serum, EDTA-plasma	Low sensitivity?	Euroimmun CCHFV-GPC and – N (Mosaic 2) IFAT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	R
ELISA	Serum, EDTA-plasma		Vector-Best IgM and IgG ELISA Vector-Best, Novosibirsk, Russia	R

6.4.7 Dengue Fever Virus (Strain 1 – 4)

Bio Safety Level 3.

6.4.7.1 PSDS

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=128&SID=5>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=129>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=130&SID=6>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=131&SID=7>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds50e-eng.php>.

http://ecdc.europa.eu/EN/HEALTHTOPICS/DENGUE_FEVER/Pages/index.aspx.

6.4.7.2 First Line Detection ‘In the Field’
Table 6-11: First Line Detection Dengue Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
RT-Recombinase polymerase amplification assay	Serum		Abd El Wahed <i>et al.</i> [1]	U
LAMP	Serum		Li <i>et al.</i> [51]	L
LAMP	Serum		Sahni <i>et al.</i> [82]	L
LAMP			Lu <i>et al.</i> [57]	L
Lateral-flow test b	Serum (?)		DENV Antigens and Antibodies for Rapid Tests http://www.biopanda-diagnostics.com/asp/products/agab_rapid.asp#dengue	R
Lateral flow test Ab	Whole blood, plasma, serum	Detects Ab against NS1	Dengue one step rapid test, Dengue NS1 http://medlab-online.com/de/dengue-fever	D
Lateral flow test Ab	See kit		DIAQUICK™ Rapid Test Anti-Dengue IgG/IgM Cassette http://www.dialab.at/product.cfm?pg_main_id=7&pg_sub_id=50&product_id=924	D
Lateral flow test Ab	Plasma, serum		Dengue NS1 Ag STRIP http://www.bio-rad.com/en-mx/sku/70700-dengue-ns1-ag-strip	D
Lateral flow test Ab	Human serum, plasma or whole blood		Panbio® Dengue Duo Cassette http://www.alere.com/en/home/product-details/panbio-dengue-duo-cassette-au.html	D
Lateral flow test Ab	Human serum, plasma or whole blood		SD BIOLINE Dengue Duo NS1 Ag + Ab Combo http://www.standardia.com/en/home/product/Rapid_Diagnostic_Test/Dengue_Duo.html	D

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6.4.7.3 Second Line Detection ‘In the Lab’

Table 6-12: Second Line Detection Dengue Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
RT-PCR LCD Array	Serum	Distinguishes subtypes	Dengue LCD Array Kit http://www.chipron.com/PDF-Downloads/Array-Products.pdf	R
rtRT-PCR	Serum, EDTA-plasma		Real Star® Dengue RT-PCR Kit 2.0 http://www.altona-diagnostics.com/realstar-product-list-EN.html	D
rtRT-PCR	Serum, EDTA-plasma		DENGUE RG quantitative Real time PCR kit (Geno-sens) https://www.laborteknik.com/en/geno-sens	D
rtRT-PCR	Serum, EDTA-plasma		TibMolBiol Kit Dengue https://www.tib-molbiol.com	R
rtRT-PCR	Serum, EDTA-plasma		Dengue genotype Real Time PCR kit http://www.sacace.com/new-dengue-virus-genotyping.htm	R
rtRT-PCR	Serum, CSF		Waggoner <i>et al.</i> [101]	L
rtRT-PCR	Serum, CSF		Gurukumar <i>et al.</i> [32]	L
rtRT-PCR	Serum, CSF		Drosten <i>et al.</i> [20]	U
rtRT-PCR	Serum, CSF		Leparc-Goffart <i>et al.</i> [50]	U
ELISA	Serum, EDTA-plasma		EUROIMMUN Anti-DENV ELISA IgG/IgA/IgM http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA	Serum		Focus Diagnostics IgG ELISAs http://www.focusdx.com/product-catalog/dxselect/elisa/ous	D
ELISA	Serum, EDTA-plasma		PLATELIA™ DENGUE NS1 AG http://www.bio-rad.com/webroot/web/pdf/inserts/CDG/en/72830_881040_EN.pdf	D

Test Method	Sample	Comment	Reference/Company	Code
ELISA	Serum, EDTA-plasma		Panbio DENV ELISA, different formats: Panbio® Dengue Duo IgM and IgG Capture ELISA Panbio® Dengue Early ELISA Panbio® Dengue Early Rapid Panbio® Dengue IgG Capture ELISA Panbio® Dengue IgG Indirect ELISA Panbio® Dengue IgM Capture ELISA http://www.alere.com/en/home/products-services/infectious/dengue-fever.html	D
ELISA	Serum, EDTA-plasma		Focus DENV IgM capture/IgG DxSelect http://www.focusdx.com/product-catalog/dxselect/elisa/ous	D
ELISA	Serum		PATHOZYME®DENGUE G Ref OD217 http://www.omegadiagnostics.pl/templates/_editor/kcfinder/upload/images/omega/OD217_1.pdf	D
ELISA	Serum		PATHOZYME®DENGUE M Capture Ref OD647 http://www.omegadiagnostics.pl/templates/_editor/kcfinder/upload/images/omega/OD647_1.pdf	D
ELISA	Serum		NovaLisa™ Dengue IgM/IgG/IgA capture http://www.novatec-id.com/de/produkte/novalisa/virologie-new/dengue/	D
ELISA	Serum, EDTA-plasma		ImmunoComb® II Dengue IgM&IgG BiSpot http://www.biolinker.com.ar/products/PDF_ALERE/Immunocomb/Dengue%20IgM%20&%20IgG%20BISpot.pdf	D

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Test Method	Sample	Comment	Reference/Company	Code
ELISA	Serum		SD Dengue IgM Capture ELISA http://www.standardia.com/en/home/product/ELISA/Anti-Dengue_IgM_Capture_ELISA.html	D
ELISA	Serum, EDTA-plasma		Human Dengue Virus IgG ELISA Kit http://www.mybiosource.com/products/ELISA-Kit/Human/Dengue-Virus-IgG/datasheet.php?products_id=580100	D
ELISA	Serum, EDTA-plasma		SERION ELISA classic Dengue Virus IgG / IgM http://www.virion-serion.de/download/flyer/Flyer_ELISA_classic_Dengue_Virus_EN.pdf	D
EIA	Serum, EDTA-plasma		Bio-Rad Platelia Dengue NS1 Ag-ELISA http://www.bio-rad.com/de-de/sku/72830-platelia-dengue-ns1-ag	D
ELISA	Serum		EUROIMMUN DENV NS1 ELISA (types 1 – 4) http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D

6.4.8 Ebola Virus

Bio Safety Level 4 (exception: strain Reston BSL 2).

6.4.8.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/ebola-eng.php>.

<http://www.cdc.gov/vhf/ebola/pdf/ebola-factsheet.pdf>.

6.4.8.2 First Line Detection ‘In the Field’

See also: http://www.who.int/medicines/ebola-treatment/emp_ebola_diagnostics/en/.

Table 6-13: First Line Detection Ebola Virus.

Test Method	Sample	Comment	Reference/Company	Code
RT-Recombinase polymerase amplification assay	Serum		Euler <i>et al.</i> [25]	U
LAMP		Inactivation techniques may interfere with the BinaxNOW test (malaria) The inactivation technique has to be validated with the test method	Kurosaki <i>et al.</i> [46]	U
ABICAP	Respiratory swab, urine, serum	Pls. contact provider for detailed information concerning sample material	ABICAP Ebola http://www.senova.de/index.php/en_gb/services/contract-development/abicap See also: http://www.diavita.de/germanneu/images/stories/Downloads/ProduktPreisliste_2008.pdf	R
Lateral flow assay	Serum		ReEBOV™ Antigen Rapid Test http://www.corgenix.com/products/infectious-disease-products/	D

6.4.8.3 Second Line Detection ‘In the Lab’

See also: http://www.who.int/medicines/ebola-treatment/emp_ebola_diagnostics/en/.

Table 6-14: Second Line Detection Ebola Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma		Gibb <i>et al.</i> [30]	U
rtRT-PCR	Serum, EDTA-plasma		Drosten <i>et al.</i> [20]	L
rtRT-PCR	Serum, EDTA-plasma		Panning <i>et al.</i> [71]	U
rtRT-PCR	Serum, EDTA-plasma		Trombley <i>et al.</i> [94]	U

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Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma	Can differentiate different EBOV strains and MARV	RealStar® Filovirus Screen RT-PCR Kit 1.0 http://www.altona-diagnostics.com/tl_files/website/downloads/RealStar_Filovirus%20Screen%20RT-PCR%20Kit%2010_CE_WEB_2014-08-29.pdf	D
rtRT-PCR	Serum, EDTA-plasma	Only for EBOV strain Zaire	Liferiver Ebola Virus (EBOV) Real Time RT-PCR Kit http://www.liferiverbiotech.com/Pages/NewsDetail.aspx?id=261	D
rtRT-PCR	Serum, EDTA-plasma		Ebola PCR kit (Reston, Zaire, Sudan, common, Ebola or Marburg virus) http://genekam.de/products/pcr-kit-for-ebola-virus-and-therapy.php	D
rtRT-PCR	Serum, EDTA-plasma		LIPSGENE® SEBOV Kit (Sudan) / ZEBOV Kit (Zaire) http://www.biocore-diagnostics.de/de/2014/02/pathogen-detection/	D
rtRT-PCR	Serum, EDTA-plasma		Ebola Zaire Real Time PCR kit http://www.sacace.com/new-ebola-zaire-virus.htm	D
rtRT-PCR	Serum, EDTA-plasma		Ebola Real Time PCR kit http://www.genesig.com/products/9654	R
rtRT-PCR	Serum, EDTA-plasma	Only for EBOV strain Zaire	Ebola PCR kit https://www.tib-molbiol.com/	R

6.4.9 Equine Encephalitis Viruses

Bio Safety Level 3.

6.4.9.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/equine-eng.php>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=136>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=520>.

<http://www.cdc.gov/EasternEquineEncephalitis/tech/factSheet.html>.

6.4.9.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.9.3 Second Line Detection ‘In the Lab’

Table 6-15: Second Line Detection Equine Encephalitis Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor	Different primer pairs for VEEV, WEEV and EEEV	Lambert <i>et al.</i> [47]	U
rtRT-PCR	Serum, EDTA-plasma, liquor	Different primer pairs for VEEV, WEEV and EEEV	Pisano <i>et al.</i> [76]	L
rtRT-PCR	Serum, EDTA-plasma, liquor		Linssen <i>et al.</i> [54]	U
rtRT-PCR	Serum, EDTA-plasma, liquor		Genesig Primer Design™ genesig Kit for Western Equine Encephalomyelitis Virus (WEEV) http://www.genesig.com/products/9451	R
rtRT-PCR	Serum, EDTA-plasma, liquor		G-Sphere Molecular Standards Eastern equine encephalitis http://www.philekorea.co.kr/kor/bbs/product.php?idx=65	R
IFAT/ELISA	Serum, EDTA-plasma	Cross reactivity of alphaviruses, exists in specialized laboratories	Merk Millipore Anti-Eastern Equine Encephalitis Antibody http://www.merckmillipore.com/DE/en/product/Anti-Eastern-Equine-Encephalitis-Antibody%2C-Virus%2C-clone-1A4B.6,MM_NF-MAB8754	R

6.4.10 Hantaviruses

Bio Safety Level 2 (e.g., Puumala virus), BSL-3 (e.g., Dobrava virus, Seoul virus, Sin Nombre Virus), BSL-4 (Andes virus).

6.4.10.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/hantavirus-eng.php>.

<http://www.cdc.gov/hantavirus/pdf/hps-fact-sheet.pdf>.

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6.4.10.2 First Line Detection ‘In the Field’

Table 6-16: First Line Detection Hantaviruses.

Test Method	Sample	Comment	Reference/Company	Code
Lateral flow test AG	Serum, plasma (heparin or EDTA) or fingertip blood serum	Cross reactivity of hantaviruses	Puumala virus – Reagent POC® Puumala virus – ReaScan® Dobrava-Hantaan virus – ReaScan® Dobrava-Hantaan virus – Reagent POC® http://www.reagent.com/en/products/infectious-diseases-zoonoses/point-care-rapid-tests/	R/D
Immunoblot	Serum	For sample material see kit instructions, two different profiles available	EUROLINE Hantavirus global profile (IgG/IgM) http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html#c508	D
Lateral flow	Serum, plasma		recomLine HantaPlus IgG recomLine HantaPlus IgM http://www.mikrogen.de/deutsch/deutschland/nc/produkte/produktuebersicht/testsystem/hantaplus-igg.html	D

6.4.10.3 Second Line Detection ‘In the Lab’

Table 6-17: Second Line Detection Hantaviruses.

Test Method	Sample	Comment	Reference/Company	Code
RT-PCR	Serum, urine		Kramski <i>et al.</i> [42]	U
rtRT-PCR	Serum, urine	Samples see kit instructions	Hanta virus RG quantitative real-time PCR kit (geno-sens) https://www.labortechnik.com/en/geno-sens	D
RT-PCR	Serum, urine	Detects Puumala, tula and dobrava virus	Essbauer <i>et al.</i> [23]	U
RT-PCR	Serum, urine	Distinguishes types by melting curve	Mossbrugger <i>et al.</i> [59]	U

Test Method	Sample	Comment	Reference/Company	Code
Immunoblot	Serum	For sample material see kit instructions, two different profiles available	EUROLINE Hantavirus global profile (IgG/IgM) http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html#c508	D
IFAT	Serum, EDTA-plasma	Cross reactivity of hantaviruses	EUROIMMUN Hantaviruses IIFT BIOCHIP (HFRS, HPS) http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
IFAT	Serum, EDTA-plasma		Hantavirus Dobrava / Hantaan Antibody IFA Hantavirus Puumala Antibody IFA Hantavirus Seoul Antibody IFA http://www.progen.de/en/topics/hantavirus.html	D
ELISA	Serum, EDTA-plasma	Cross reactivity of hantaviruses	EUROIMMUN Anti-Hantavirus Pool 1 "Eurasia" ELISA IgG/IgM, avidity http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	
ELISA	Serum, EDTA-plasma	Cross reactivity of hantaviruses	EUROIMMUN Anti-Hantavirus Pool 2 "America" ELISA IgG/IgM, avidity http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	
ELISA	Serum	Cross reactivity of hantaviruses	Hantavirus Puumala IgG/IgM ELISA http://www.ibl-international.com/media/catalog/product/R/E/RE57461_IFU_en_Hanta_puumala_IgG_IgM_2014-05_sym3.pdf	D
ELISA	Serum	Cross reactivity of hantaviruses	Hantavirus Dobrava IgG/IgM ELISA http://www.ibl-international.com/media/catalog/product/R/E/RE57471_IFU_en_Hanta_Dobrava_IgG_IgM_V2013_05_sym3.pdf	D

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Test Method	Sample	Comment	Reference/Company	Code
ELISA	Serum	Cross reactivity of hantaviruses	Hantavirus Hantaan IgG/IgM ELISA http://www.ibl-international.com/media/catalog/product/R/E/RE57451_IFU_en_Hanta_hantaan_IgG_IgM_2014-05_sym3.pdf	D
EIA	Serum		Reagent DOBRAVA-HANTAAN IgG EIA http://reagent.ru/www/en/products/pdf/instructions/Reagent-DOBRAVA-HANTAAN-IgG-EIA-Ver-1.1-ENG.pdf	D
ELISA	Serum		Focus Hantavirus IgM/IgG, DXSelect™ http://www.focusdx.com/product-catalog/dxselect/elisa/ous	D
EIA	Serum, EDTA-plasma	Cross reactivity of hantaviruses	REAGENA(Puumala IgM and IgG EIA http://www.reagent.com/en/products/infectious-diseases-zoonoses/eia/puumala/	D
ELISA	Serum, EDTA-plasma	Cross reactivity of hantaviruses	Hantavirus Dobrava / Hantaan IgG/IgM ELISA Hantavirus Puumala IgG/IgM ELISA http://www.progen.de/en/topics/hantavirus.html	D

6.4.11 Hendra Virus

Bio Safety Level 4.

6.4.11.1 PSDS

<http://www.cdc.gov/vhf/hendra/resources/Hendra-FactSheet.pdf>.

6.4.11.2 First Line Detection 'In the Field'

There are currently no first line detection methods available.

6.4.11.3 Second Line Detection ‘In the Lab’

Table 6-18: Second Line Detection Hendra Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR			Hendra and Nipah virus diseases OIE Terrestrial Manual [63]	L
rtRT-PCR			Smith <i>et al.</i> [89]	L
Antigen capture ELISA		Exists in specialized laboratories	Chiang <i>et al.</i> [12]	L
Antigen capture ELISA		Exists in specialized laboratories	Crameri <i>et al.</i> [17]	L

6.4.12 Hepatitis E Virus

Bio Safety Level 3(**).

*Bio Safety Level 3 ** has in general the same basic laboratory equipment as Bio Safety Level 2. In addition, there has to be a separate room for changing Personal Protection Equipment which has to be selected according to the work and techniques used. The laboratory waste has to be collected separately and the waste water has to be collected and autoclaved.*

6.4.12.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/hepe-eng.php>.

6.4.12.2 First Line Detection ‘In the Field’

Table 6-19: First Line Detection Hepatitis E Virus.

Test Method	Sample	Comment	Reference/Company	Code
Lateral flow test			Abravanel <i>et al.</i> [3]	L

6.4.12.3 Second Line Detection ‘In the Lab’

Table 6-20: Second Line Detection Hepatitis E Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	See kit instructions		Ceeram Diagnostics Hepatitis E Virus Assay http://www.ceeramtools.com/kit-hepatitis-e-virus-kit.html	nk

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Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, urine		Altona Diagnostics RealStar® HEV RT-PCR Kit 1.0 http://www.altona-diagnostics.com/realstar-product-list-EN.html	D
rtRT-PCR	Serum, urine	Samples see kit instructions	HEV RG quantitative real-time PCR kit (geno-sens) https://www.labortechnik.com/en/geno-sens	D
rtRT-PCR			Jothikumar <i>et al.</i> [37]	L
rtRT-PCR			Johne <i>et al.</i> [36]	L
ELISA	Serum, plasma		Bioelisa HEV IgG http://www.biokit.com/productos/reagents/bioelisa/hepatitis/bioelisa-hev-igg.aspx	D
EIA	Serum, plasma		Genelabs [Singapore] HEV IgG EIA kits	D

6.4.13 Herpes B Virus

Bio Safety Level 3.

6.4.13.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/herpes-cerco-eng.php>.

6.4.13.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.13.3 Second Line Detection ‘In the Lab’

Table 6-21: Second Line Detection Herpes B Virus.

Test Method	Sample	Comment	Reference/Company	Code
ELISA			Katz <i>et al.</i> [38]	L
ELISA			Yamamoto <i>et al.</i> [107]	L
PCR			Oya <i>et al.</i> [68]	

6.4.14 Influenza Viruses (Zoonotic)

Bio Safety Level 3 (H5 HPA, H7 HPA; H1N1, H7N9, H2N2, H5N1), BSL-2 (others).

6.4.14.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/influenza-grippe-a-eng.php>.

6.4.14.2 First Line Detection ‘In the Field’

Table 6-22: First Line Detection Zoonotic Influenza Virus.

Test Method	Sample	Comment	Reference/Company	Code
Lateral flow test	Nasal swab, nasopharyngeal swab, nasal aspirate/nasal wash		Dialab DIAQUICK Rapid Test H5 AIV Ag Cassette http://www.dialab.hu/dok/119/Dialab_gyorstesztekt.pdf	D
Lateral flow test	Nasal swab, nasopharyngeal swab, nasal aspirate/nasal wash		QuickVue® Influenza A+B Kit https://www.quidel.com/immunoassays/rapid-influenza-tests/quickvue-influenza-test	D
Lateral flow test	Nasopharyngeal (NP) swab, nasal swab samples and nasal wash/aspirate		BinaxNOW® Influenza A & B Test http://www.alere.com/en/home/product-details/binaxnow-influenza-a-and-b.html?c=US	D
Proximity ligation assay			Schlingemann <i>et al.</i> [86]	L

6.4.14.3 Second Line Detection ‘In the Lab’

Table 6-23: Second Line Detection Zoonotic Influenza Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Nasopharyngeal (NP) swab, nasal swab samples and nasal wash/aspirate		TIB MolBiol Kit Influenza A/B/H5 and others https://www.tib-molbiol.com	R
rtRT-PCR	Nasopharyngeal (NP) swab, nasal swab samples and nasal wash/aspirate		VLA TaqMan® Influenza A/H5/H7 Detection Kits https://www3.appliedbiosystems.com/cms/groups/applied_markets_marketing/documents/generaldocuments/cms_094231.pdf	D
rtRT-PCR	Nasopharyngeal (NP) swab, nasal swab samples and nasal wash/aspirate		artus Influenza LC/TM/RG RT-PCR kit (24/96) RUO https://www.qiagen.com/ca/shop/detection-solutions/human-pathogens/artus-sars-rg-rt-pcr-kit-ruo/	D

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Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Nasopharyngeal (NP) swab, nasal swab samples and nasal wash/aspirate		TaqMan® Avian Influenza Virus (AIV-M) Reagents https://www.thermofisher.com/order/catalog/product/4405543	nk
rtRT-PCR	Nasopharyngeal (NP) swab, nasal swab samples and nasal wash/aspirate		Influenza A,B Real Time PCR kit http://www.sacace.com/?id=0&sid=1&q=influenza	nk
rtRT-PCR	Nasopharyngeal (NP) swab, nasal swab samples and nasal wash/aspirate		Real Star® Influenza RT-PCR Kit 1.0 (human A & B) http://www.altona-diagnostics.com/realstar-product-list-EN.html	D
rtRT-PCR	See kit instructions for details		H5N1 (bird flu) RG quantitative real-time PCR kit (geno-sens) https://www.labortechnik.com/en/geno-sens	D
rtRT-PCR			Chen <i>et al.</i> [11]	L
rtRT-PCR			Tsukamoto <i>et al.</i> [96]	L
rtRT-PCR			Tsukamoto <i>et al.</i> [95]	L
rtRT-PCR			Nagajaran <i>et al.</i> [62]	L
rtRT-PCR			De Santis <i>et al.</i> [18]	L
IFAT	Serum, EDTA-plasma		Bartels® Viral Respiratory Screening and Identification Kit Bartels® Viral Respiratory Screening and Identification Kit: Influenza A Antibody Bartels® Viral Respiratory Screening and Identification Kit: Influenza B Antibody http://www.trinitybiotech.com/products/vrk-influenza-b-ab/	R
IFAT	Serum, EDTA-plasma		EUROIMMUN Influenza A/B IgA/IgG/IgM IIFT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA	Human serum or EDTA plasma		Anti-Influenza A/B Virus Pool ELISA (IgG) http://www.euroimmun.us/package-inserts/Infectious/ELISA/ELISA/EI_2691-1G_A_US_C03.pdf	D

Test Method	Sample	Comment	Reference/Company	Code
IFAT	Serum, EDTA-plasma		EUROIMMUN Anti-Influenza A, B, or AB Pool ELISA, IgA/IgG/IgM http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA	Serum and plasma		ID Screen® Influenza A Antibody Competition Multi-species http://www.id-vet.com/produit/id-screen-influenza-a-antibody-competition-multi-species/	
	Serum		IDEXX Influenza A Ab Test https://www.idexx.com/pdf/en_us/livestock-poultry/influenza-a-ab-test-sheet.pdf	
ELISA	Serum and plasma		SERION ELISA classic Influenza A Virus IgA / IgG / IgM http://www.virion-serion.de/download/flyer/Flyer_ELISA_classic_Influenza_Virus_EN.pdf	D
ELISA	Serum and plasma		Influenza B Virus IgA / IgG / IgM http://www.virion-serion.de/download/flyer/Flyer_ELISA_classic_Influenza_Virus_EN.pdf	D
ELISA	Serum and plasma		Influenza A IgA ELISA Influenza A IgM/G ELISA Influenza B IgA ELISA Influenza B IgM/G ELISA http://www.sekisuidiagnostics.com/products/category/3-virotech-products	D
ELISA	Serum and plasma		NovaLisa™ Influenza A IgA/IgM/IgG http://www.novatec-id.com/de/produkte/novalisa/virologie-new/influenza/	D

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Test Method	Sample	Comment	Reference/Company	Code
ELISA	Serum and plasma		NovaLisa™ Influenza B IgA/IgM/IgG http://www.novatec-id.com/de/produkte/novalisa/virologie-new/influenza/	D
Complement fixation test	See kit instructions		SERION CFT http://www.virion-serion.de/en/products/serion-cft.html	nk
Complement fixation test/EIA			Influenza A/B http://www.diesse.it/en/Products/dettaglio:245/	nk

6.4.15 Japanese Encephalitis Virus

Bio Safety Level 3.

6.4.15.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds173e-eng.php>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=208>.

6.4.15.2 First Line Detection ‘In the Field’

Table 6-24: First Line Detection Japanese Encephalitis Virus.

Test Method	Sample	Comment	Reference/Company	Code
LAMP			Liu <i>et al.</i> [55]	L
LAMP			Tian <i>et al.</i> [93]	L
LAMP	Serum		Li <i>et al.</i> [51]	R

6.4.15.3 Second Line Detection ‘In the Lab’

Table 6-25: Second Line Detection Japanese Encephalitis Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor		Liferiver Japanese Encephalitis Virus (JEV) Real Time RT-PCR Kit http://www.liferiverbiotech.com/Pages/Product/ProductDetail.aspx?productId=411	D

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor		Genome Diagnostic JEV RG quantitative Real time PCR kit http://www.genomediagnostics.co.in/	D
rtRT-PCR	Serum, EDTA-plasma, liquor		Chuang <i>et al.</i> [13]	L
rtRT-PCR	Serum, EDTA-plasma, liquor		Jeong <i>et al.</i> [35]	L
rtRT-PCR	Serum, EDTA-plasma, liquor		Huang <i>et al.</i> [34]	L
rtRT-PCR	Serum, EDT-plasma, liquor	Samples see kit instructions	JEV RG quantitative real-time PCR kit (geno-sens) https://www.labortechnik.com/en/geno-sens	D
ELISA	Serum, EDTA-plasma	There might be cross-reaction with other flaviviruses	Panbio Japanese Encephalitis - Dengue IgM combo http://www.panbio.com/download/PB0110_JE_Dengue%20Combo%20IgM%20ELISA.pdf	D
ELISA	Serum	There might be cross-reaction with other flaviviruses	JE Detect™ IgG ELISA for Japanese Encephalitis http://www.inbios.com/elisas/je-detect-igg	D (CE certified)
ELISA	Serum	There might be cross-reaction with other flaviviruses	JE Detect™ IgM Capture ELISA for Japanese Encephalitis http://www.inbios.com/elisas/je-detect-igm	D (CE certified)
FAT	Serum, EDTA-plasma	There might be cross-reaction with other flaviviruses	Euroimmun JEV IgG/IgM IIFT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D (CE certified)
IFAT	Serum, EDTA-plasma	There might be cross-reaction with other flaviviruses	Euroimmun Flavi-Mosaik IgG/IgM IIFT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA	Serum, EDTA-plasma	There might be cross-reaction with other flaviviruses	Euroimmun anti-JEV ELISA IgG/IgM http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	

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6.4.16 Kyasanur Forest Disease Virus

Bio Safety Level 3.

6.4.16.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/kyasanur-eng.php>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=252>.

6.4.16.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.16.3 Second Line Detection ‘In the Lab’

Table 6-26: Second Line Detection Kyasanur Forest Disease Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor		Mourya <i>et al.</i> [60]	L
Ab		There might be cross-reaction with other flaviviruses, exists in specialized laboratories		

6.4.17 Lassa Fever Virus

Bio Safety Level 4.

6.4.17.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/lassa-eng.php>.

http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/fact_sheets/lassa_fever_fact_sheet.pdf.

6.4.17.2 First Line Detection ‘In the Field’

Table 6-27: First Line Detection Lassa Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
LAMP			Fukuma <i>et al.</i> [29]	L
Lateral flow assay	Serum		Corgenix ReLASV® Antigen Rapid Test for Diagnosis of Lassa Fever http://www.corgenix.com/news-releases/corgenix-gains-ce-mark-for-relasv-antigen-rapid-test-for-diagnosis-of-lassa-fever/	D

6.4.17.3 Second Line Detection ‘In the Lab’

Table 6-28: Second Line Detection Lassa Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-blood	Strains change quickly, has eventually be adapted	Ölschläger <i>et al.</i> [65]	U
rtRT-PCR	Serum, EDTA-blood	Strains change quickly, has eventually be adapted	Ölschläger <i>et al.</i> [66]	U
rtRT-PCR	Serum, EDTA-blood	Strains change quickly, has eventually be adapted	Drosten <i>et al.</i> [20]	U
rtRT-PCR	Serum, EDTA-blood	Strains change quickly, has eventually be adapted	Vieth <i>et al.</i> [99]	U
rtRT-PCR	Serum, EDTA-blood	Strains change quickly, has eventually be adapted	Lassa fever virus nucleic acid assay kit http://www.cosmosbiomedical.com/pcr.php	R
Ab		Exists in specialized laboratories		

6.4.18 Lymphocytic Choriomeningitis Virus

Bio Safety Level 2 (neurotropic strains BSL-3).

6.4.18.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/lymp-cho-eng.php>.

http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/Fact_Sheets/Lymphocytic_Choriomeningitis_Fact_Sheet.pdf.

6.4.18.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.18.3 Second Line Detection ‘In the Lab’

Table 6-29: Second Line Detection Lymphocytic Choriomeningitis Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR			Knust <i>et al.</i> [41]	L
rtRT-PCR			Cordey <i>et al.</i> [15]	L
Ab		Exists in specialized laboratories		

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6.4.19 Marburg Virus

Bio Safety Level 4.

6.4.19.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/marburg-eng.php>.

http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/Fact_Sheets/Marburg%20Hemorrhagic%20Fever%20Fact%20Sheet.pdf.

6.4.19.2 First Line Detection ‘In the Field’

Table 6-30: First Line Detection Marburg Virus.

Test Method	Sample	Comment	Reference/Company	Code
RT-Recombinase polymerase amplification assay	Serum		Euler <i>et al.</i> [25]	L
LAMP			Kurosaki <i>et al.</i> [45]	L
ABICAP	Respiratory swab, urine, serum	Pls. contact provider for detailed information concerning sample material	Abicap Ebola http://www.senova.de/index.php/en_gb/services/contract-development/abicap See also: http://www.diavita.de/germanneu/images/stories/Downloads/ProduktPreisliste_2008.pdf	R

6.4.19.3 Second Line Detection ‘In the Lab’

Table 6-31: Second Line Detection Marburg Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma	Can differentiate different EBOV strains and MARV	Altona diagnostics RealStar® Filovirus Screen RT-PCR Kit 1.0 http://www.altona-diagnostics.com/realstar-product-list-EN.html	nk
rtRT-PCR	Serum, EDTA-plasma		Liferiver Marburg Virus (MBV) Real Time RT-PCR Kit http://www.liferiverbiotech.com/Pages/Product/ProductDetail.aspx?productId=429	D

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma		Ogawa <i>et al.</i> [64]	L
rtRT-PCR	Serum, EDTA-plasma		Drosten <i>et al.</i> [20]	L
rtRT-PCR	Serum, EDTA-plasma		Trombley <i>et al.</i> [94]	U
rtRT-PCR	Serum, EDTA-plasma		Weidmann <i>et al.</i> [102]	U
Ab		Exists in specialized laboratories		

6.4.20 Nipah Virus

Bio Safety Level 4.

6.4.20.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/nipah-eng.php>.

<http://www.cdc.gov/vhf/nipah/resources/Nipah-FactSheet.pdf>.

6.4.20.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.20.3 Second Line Detection ‘In the Lab’

Table 6-32: Second Line Detection Nipah Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma		Hendra and Nipah virus diseases OIE Terrestrial Manual [63]	L
Ab		Exists in specialized laboratories		

6.4.21 O’Nyong-Nyong Virus

Bio Safety Level 2.

6.4.21.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds130e-eng.php>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=348>.

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6.4.21.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.21.3 Second Line Detection ‘In the Lab’

Table 6-33: Second Line Detection O’Nyong-Nyong Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma		Smith <i>et al.</i> [88]	L
rtRT-PCR	Serum, EDTA-plasma		Plaskon <i>et al.</i> [77]	L
Ab		There might be cross-reaction with other alphaviruses, exists in specialized laboratories		

6.4.22 Omsk Hemorrhagic Fever Virus

Bio Safety Level 3.

6.4.22.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/omsk-eng.php>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=347>.

6.4.22.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.22.3 Second Line Detection ‘In the Lab’

Table 6-34: Second Line Detection Omsk Hemorrhagic Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor		Pang <i>et al.</i> [69]	L
Ab		There might be cross-reaction with other flaviviruses, exists in specialized laboratories		

6.4.23 Orthopox Viruses (Zoonotic)

Bio Safety Level 2 (Monkey pox virus BSL-3).

6.4.23.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/orthopoxvirus-eng.php>.

<http://stacks.cdc.gov/view/cdc/26229>.

6.4.23.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.23.3 Second Line Detection ‘In the Lab’

Table 6-35: Second Line Detection Zoonotic Orthopox Viruses.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Skin bioptate, rush		Pulford <i>et al.</i> [80]	U
rtRT-PCR	Skin bioptate, rush		Euler <i>et al.</i> [25]	L
rtRT-PCR	Skin bioptate, rush		Sofi Ibrahim [90]	L
rtRT-PCR	Skin bioptate, rush		Panning <i>et al.</i> [70]	U
rtRT-PCR	Skin bioptate, rush	For monkeypox	Li <i>et al.</i> [52]	L
Ab		Exists in specialized laboratories		

6.4.24 Rabies Virus

Bio Safety Level 3.

6.4.24.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/rab-eng.php>.

DETECTION METHODS

6.4.24.2 First Line Detection ‘In the Field’

Table 6-36: First Line Detection Rabies Virus.

Test Method	Sample	Comment	Reference/Company	Code
Rapid immunodiagnostic assay	RIDA works well on brain samples (post mortem diagnosis in dogs) but is only validated for the most prevalent strain (classical rabies virus, genotype 1)		Yang <i>et al.</i> [108]	L

6.4.24.3 Second Line Detection ‘In the Lab’

Table 6-37: Second Line Detection Rabies Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor		Wacharapluesadee <i>et al.</i> [100]	L
rtRT-PCR	Serum, EDTA-plasma, liquor		Yang <i>et al.</i> [108]	L
ELISA	Serum, plasma		PLATELIA Rabies II Kit http://www.bio-rad.com/en-us/sku/3550180-platelia-rabies-ii-kit-ad-usum-veterinarium	R
Ab		Exists in specialized laboratories		

6.4.25 Rift Valley Fever Virus

Bio Safety Level 3 (exception: RFV strain MP12 BSL2).

6.4.25.1 PSDS

http://ecdc.europa.eu/en/healthtopics/rift_valley_fever/Pages/index.aspx

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=397>.

http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/Fact_Sheets/Rift%20Valley%20Fever%20Fact%20Sheet.pdf.

6.4.25.2 First Line Detection ‘In the Field’

Table 6-38: First Line Detection Rift Valley Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
RT-Recombinase polymerase amplification assay	Serum		Euler <i>et al.</i> [26]	U
LAMP			Le Roux <i>et al.</i> [49]	L

6.4.25.3 Second Line Detection ‘In the Lab’

Table 6-39: Second Line Detection Rift Valley Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-Plasma		TibmolBiol Rift valley fever virus PCR http://www.tib-molbiol.de/de/	R
rtRT-PCR	Serum, EDTA-Plasma		Wilson <i>et al.</i> , 2013 [106]	L
rtRT-PCR	Serum, EDTA-Plasma		Mwaengo <i>et al.</i> [61]	L
rtRT-PCR	Serum, EDTA-Plasma		Grobbelaar <i>et al.</i> [31]	L
rtRT-PCR	Serum, EDTA-Plasma		Drosten <i>et al.</i> [20]	L
rtRT-PCR	Serum, EDTA-Plasma		Bird <i>et al.</i> [7]	U
IFAT	Serum, EDTA-Plasma		EUROIMMUN RVFV IIFT IgG/IgM http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D, U
Ab		Exists in specialized laboratories		

6.4.26 Sandfly Fever Virus (e.g., Sandfly Sicilian Virus, Sandfly Naples Virus, Toscana Virus, Sandfly Turkey Virus)

Bio Safety Level 2.

DETECTION METHODS

6.4.26.1 PSDS

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=417>.

6.4.26.2 First Line Detection ‘In the Field’

Table 6-40: First Line Detection Sandfly Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
LAMP	Serum		Xu <i>et al.</i> [106]	L
Lateral flow assay IgG/IgM	Serum	Serotype Sicilian and Toscana virus	MIKROGEN (Western blot recomLine Bunyavirus IgG/IgM) http://www.mikrogen.de/deutsch/deutschland/produkte/produktuebersicht/tecosystem/hantaplus-igg.html	nk

6.4.26.3 Second Line Detection ‘In the Lab’

Table 6-41: Second Line Detection Sandfly Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor		Weidmann <i>et al.</i> [103]	L
rtRT-PCR	Serum, EDTA-plasma, liquor	Detects only TOSV	Pérez-Ruiz <i>et al.</i> [74]	U
ELISA	Serum, EDTA-plasma	There might be cross-reaction between different sandfly virus types	Chorus TOSCANA VIRUS IgG (Sandfly Fever Virus) Chorus TOSCANA VIRUS IgM (Sandfly Fever Virus) http://www.diesse.it/en/Product/s/dettaglio:252/	nk
Blot	Serum, plasma		recomLine Bunyavirus IgG/IgM https://www.qedbio.com/v/pdf/PIRLBUE.pdf	U, D
IFAT	Serum, EDTA-plasma	There might be cross-reaction between different sandfly virus types	Euroimmun Phlebo Biochip IIFT (sandfly viruses) http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D

Test Method	Sample	Comment	Reference/Company	Code
IFAT	Serum	There might be cross-reaction between different sandfly virus types	“Sandfly fever virus Mosaic 1” types Sicilian, Naples, Toscana, Cyprus https://www.euroimmun.de/produkte/indikationen/infektions-serologie/tropenkrankheiten.html#c506	U, R

6.4.27 Sindbis Virus

Bio Safety Level 2.

6.4.27.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds140e-eng.php>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=444>.

6.4.27.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.4.27.3 Second Line Detection ‘In the Lab’

Table 6-42: Second Line Detection Sindbis Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma		Sane <i>et al.</i> [84]	L
rtRT-PCR	Serum, EDTA-plasma			L
IFAT	Serum, EDTA-plasma	There might be cross-reaction with other alphaviruses	Euroimmun Sindbis virus IIFT https://www.euroimmun.de/produkte/indikationen/infektions-serologie/tropenkrankheiten.html#c506	R (?)
Ab		There might be cross-reaction with other alphaviruses, exists in specialized laboratories		

6.4.28 Tick-Borne Encephalitis Virus

Bio Safety Level 3 (western type 3**).

DETECTION METHODS

*Bio Safety Level 3 ** has in general the same basic laboratory equipment as Bio Safety Level 2. In addition, there has to be a separate room for changing Personal Protection Equipment which has to be selected according to the work and techniques used. The laboratory waste has to be collected separately and the waste water has to be collected and autoclaved.*

6.4.28.1 PSDS

<http://ecdc.europa.eu/en/healthtopics/vectors/world-health-day-2014/documents/factsheet-tick-borne-encephalitis.pdf>.

http://www.cdc.gov/ncidod/dvrd/spb/pdf/Tick-borne_Encephalitis_Fact_Sheet.pdf.

6.4.28.2 First Line Detection ‘In the Field’

Table 6-43: First Line Detection Tick-Borne Encephalitis Virus.

Test Method	Sample	Comment	Reference/Company	Code
LAMP			Hayasaka <i>et al.</i> [33]	L
Lateral flow assay	Serum		ReaScan® TBE IgM (Rapid Test) http://www.reagen.com/en/products/infectious-diseases-zoonoses/point-care-rapid-tests/tbe-virus-reascan/	nk

6.4.28.3 Second Line Detection ‘In the Lab’

Table 6-44: Second Line Detection Tick-Borne Encephalitis Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Liquor, serum, EDT-plasma		Schwaiger & Cassinotti [87]	U
rtRT-PCR	Liquor, serum, EDT-plasma		Tick borne diseases (also TBEV) Real Time PCR kit http://www.sacace.com	R
rtRT-PCR	Liquor, serum, EDT-plasma		Fajs <i>et al.</i> [28]	L
rtRT-PCR	Serum, EDTA-plasma		Donoso Mantke <i>et al.</i> [19]	L
ELISA	Serum, EDTA-plasma		EIA TBE Virus IgG/IgM http://www.testlinecd.com/en/vyhledavani-eshop/?q=tbe&submit=GO!	nk

Test Method	Sample	Comment	Reference/Company	Code
IFAT	Serum, EDTA-plasma	there might be cross-reaction with other flaviviruses, check vaccination status with TBEV, YFV	Euroimmun TBEV IgA/IgG/IgM IIFT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
IFAT	Serum, EDTA-plasma	There might be cross-reaction with other flaviviruses, check vaccination status with TBEV, YFV	Euroimmun Flavivirus Mosaik IIFT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA	Serum	There might be cross-reaction with other flaviviruses, check vaccination status with TBEV, YFV	Euroimmun Anti-TBEV ELISA IgG/IgM, avidity http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA	Serum	There might be cross-reaction with other flaviviruses, check vaccination status with TBEV, YFV	Euroimmun anti-TBEV ELISA http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA	Serum, liquor, EDTA-plasma	There might be cross-reaction with other flaviviruses, check vaccination status with TBEV, YFV	SERION ELISA classic FSME virus IgG/IgM http://www.virion-serion.de/download/flyer/Flyer_ELISA_classic_FSME_Virus_DE.pdf	D
ELISA	Human serum, plasma and cerebrospinal fluid		IMMUNOZYM® FSME IgG All species http://www.progen.de/en/diagnostics/infectious-disease/tick-borne-encephalitis.html	D
ELISA	Human serum, plasma and cerebrospinal fluid		IMMUNOZYM® FSME (TBE) IgG/IgM http://www.progen.de/en/diagnostics/infectious-disease/tick-borne-encephalitis.html	D
ELISA	Serum, plasma		FSME / TBE ELISA IgG / IgM Test Kit http://www.sekisuidiagnostics.com/products/506-fsme-tbe-igg-igm-elisa-virotech	D

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
ELISA	Serum, plasma		NovaLisa™ TBEV IgM/IgG http://www.novatec-id.com/de/produkte/novalisa/virologie-new/fsme-virus/	D
ELISA	Serum, plasma or CSF		TBE virus (FSME) IgG ELISA http://www.ibl-international.com/media/catalog/product/R/E/RE57401_IFU_en_TBEV_FSME_IgG_ELISA_2014-05_sym3.pdf	D
ELISA	Serum, plasma or CSF		TBE virus (FSME) IgM ELISA http://www.ibl-international.com/media/catalog/product/f/i/file_147.pdf	D
ELISA	Serum		Reagent TBE IgM EIA Reagent TBE IgG EIA http://reagent.ru/eNewsletter4/en/2013/TBElaunch/Reagent_EIA_TBE_IgG_IgM_ENG_1page.pdf	D
ELISA	Serum, EDTA-plasma	There might be cross-reaction with other flaviviruses, check vaccination status with TBEV, YFV	Progen IMMUNOZYME® FSME IgG/IgM http://www.progen.de/de/diagnostik/infektionserkrankungen/fsme.html	D

6.4.29 West Nile Virus

Bio Safety Level 3.

6.4.29.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/wnv-vno-eng.php>.

<https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=519>.

http://www.cdc.gov/westnile/resources/pdfs/wnvFactsheet_508.pdf.

6.4.29.2 First Line Detection ‘In the Field’

Table 6-45: First Line Detection West Nile Virus.

Test Method	Sample	Comment	Reference/Company	Code
LAMP	Serum		Li <i>et al.</i> [51]	R

6.4.29.3 Second Line Detection ‘In the Lab’

Table 6-46: Second Line Detection West Nile Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma, liquor	Pls. read kit instructions for samples and platforms	RealStar® WNV RT-PCR Kit 1.0 http://www.altona-diagnostics.com/realstar-product-list-EN.html	D
rtRT-PCR	Serum, EDTA-plasma, liquor	Pls. read kit instructions for samples and platforms	WNV PCR http://www.tibmolbiol.de	R
rtRT-PCR	Serum, EDTA-plasma, liquor	Pls. read kit instructions for samples and platforms	Qiagen – artus WNV LC RT-PCR Kit https://www.qiagen.com/ca/shop/detection-solutions/human-pathogens/artus-sars-rg-rt-pcr-kit-ruo/	D
rtRT-PCR	Serum, EDTA-plasma, liquor	Pls. read kit instructions for samples and platforms	Altona diagnostics Real Star® WNV RT-PCR Kit 1.0 http://www.altona-diagnostics.com/realstar-product-list-EN.html	D
rtRT-PCR	Serum, EDTA-plasma, liquor	Pls. read kit instructions for samples and platforms	West Nile virus Real Time PCR kit http://www.sacace.com	R
rtRT-PCR	Serum, EDT-plasma, liquor	Samples see kit instructions	WNV RG quantitative real-time PCR kit (geno-sens) https://www.labortechnik.com/en/geno-sens	D
rtRT-PCR	Serum, EDTA-plasma, liquor		Lanciotti <i>et al.</i> [48]	U
rtRT-PCR	Serum, EDTA-plasma, liquor		Faggioni <i>et al.</i> [27]	U
rtRT-PCR	Serum, EDTA-plasma, liquor		Kumar <i>et al.</i> [44]	L
rtRT-PCR	Serum, EDTA-plasma, liquor		Lim <i>et al.</i> [53]	L
IFAT	Serum, EDTA-plasma	There might be cross-reaction with other flaviviruses	Euroimmun WNV IgG/IgM/avidity IIFT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
ELISA	Serum	There might be cross-reaction with other flaviviruses	Euroimmun anti-WNV ELISA IgG/IgM, avidity http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D
ELISA	Serum	There might be cross-reaction with other flaviviruses	Panbio (WNV ELISA) http://www.panbio.com/download/PB0057.pdf	D
ELISA	Serum	There might be cross-reaction with other flaviviruses	Focus WNV IgM capture / IgG DxSelect™ http://www.focusdx.com/product-catalog/dxselect/elisa/ous	D
ELISA	Serum	There might be cross-reaction with other flaviviruses	SERION ELISA classic West Nile Virus IgG/IgM http://www.virion-serion.de/produkte/serion-elisa-classic/west-nile-virus.html	D
ELISA	Serum	There might be cross-reaction with other flaviviruses	West Nile Detect™ IgM Capture ELISA http://www.inbios.com/elisas/west-nile-detect-igm	D

6.4.30 Yellow Fever Virus

Bio Safety Level 3.

6.4.30.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/yfv-vfj-eng.php>.

<https://wwwn.cdc.gov/arbovat/VirusDetails.aspx?ID=531>.

6.4.30.2 First Line Detection ‘In the Field’

Table 6-47: First Line Detection Yellow Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
RT-Recombinase polymerase amplification assay	Serum		Escadafal <i>et al.</i> [22]	L

6.4.30.3 Second Line Detection ‘In the Lab’

Table 6-48: Second Line Detection Yellow Fever Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-pCR	Serum, EDTA-plasma, liquor		Bae <i>et al.</i> [6]	L
rtRT-pCR	Serum, EDTA-plasma, liquor		Mantel <i>et al.</i> [58]	U
rtRT-pCR	Serum, EDTA-plasma, liquor		Sanchez-Seco <i>et al.</i> [83]	L
rtRT-pCR	Serum, EDTA-plasma, liquor		Scaramozzino <i>et al.</i> [85]	L
rtRT-pCR	Serum, EDTA-plasma, liquor		Charlier <i>et al.</i> [10]	L
rtRT-pCR	Serum, EDTA-plasma, liquor		Drosten <i>et al.</i> [20]	U
IFAT	Serum, EDTA-plasma	There might be cross-reaction with other flaviviruses, check vaccination status with TBEV, YFV	Euroimmun anti-YFV IgG/IgM IIFT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	D

6.4.31 Middle East Respiratory Syndrome Coronavirus (MERS)

Bio Safety Level 3.

6.4.31.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/advi-avis/hce-che-2013-01-22-eng.php>.

6.4.31.2 First Line Detection ‘In the Field’

Table 6-49: First Line Detection Middle East Respiratory Virus.

Test Method	Sample	Comment	Reference/Company	Code
RT-Recombinase polymerase amplification assay	Serum		Abd El Wahed <i>et al.</i> [2]	L

DETECTION METHODS

6.4.31.3 Second Line Detection ‘In the Lab’

Table 6-50: Second Line Detection Middle East Respiratory Virus.

Test Method	Sample	Comment	Reference/Company	Code
rtRT-PCR	Serum, EDTA-plasma		Altona diagnostics Real Star [®] MERS-CoV RT-PCR Kit 1.0 http://www.altona-diagnostics.com/realstar-product-list-EN.html	
rtRT-PCR			Corman <i>et al.</i> [16]	U
IFAT	Serum		EUROIMMUN MERS-CoV IgG/IgM IIFT http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	R
ELISA	Serum		EUROIMMUN MERS-CoV ELISA IgG http://www.euroimmun.com/produkte/indikationen/infektions-serologie/tropenkrankheiten.html	R
Ab		Exists in specialized laboratories		

6.5 DETECTION METHODS OF BACTERIA

6.5.1 *Anaplasma Phagocytophilum*

Biosafety Level 2.

6.5.1.1 PSDS

See Annex D.

6.5.1.2 First Line Detection ‘In the Field’

Table 6-51: First Line Detection *Anaplasma Phagocytophilum*.

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay	Blood, liquor, serum		Bionote IDEXX (Ab dog) http://www.idexx.de/smallanimal/inhouse/snap/4dx.html	R

Test Method	Sample	Comment	Reference/Company	Code
Direct microscopy of blood smear	Blood	Experienced personnel needed		U

6.5.1.3 Second Line Detection ‘In the Lab’

Table 6-52: Second Line Detection *Anaplasma Phagocytophilum*.

Test Method	Sample	Comment	Reference/Company	Code
Culture		Cell culture, HL60 ISE6, IDE8, THP1 Difficult Experienced personnel needed		L
Microscopy	Blood smear	Giemsa stain or Diff-Quick of blood smear G-, small		U
ELISA	Serum	Only for dogs <i>Anaplasma equi</i> for horses might be applicable (validation running)	http://www.vet.euroimmun.de/fileadmin/vet_euroimmun/pdf/EI_220mC_D_UK_A.pdf	R
IF	Serum plasma	Canine and equine serum or plasma	http://www.fullerlabs.com/files/9813/4202/4464/EEX-120-English.pdf	IVD
PCR	Blood		Adiavet-BioMerieux http://www.biomerieux-industry.com/veterinary-diagnostics/anaplasma-phagocytophilum	N/A

6.5.2 *Bacillus Anthracis*

Biosafety Level 3.

6.5.2.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds12e-eng.php>.

DETECTION METHODS

6.5.2.2 First Line Detection ‘In the Field’

Table 6-53: First Line Detection *Bacillus Anthracis*.

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay	Blood, tissue, culture		Tetracore http://tetracore.com/bacillus-anthraxis-detection/index.html Miprolab http://www.miprolab.com/fileadmin/Dateiablage/PDF/MF512-001-02_PriceList-miPROTECT_Test_system.pdf	IVD, R
Microscopy	Blood, tissue	Direct microscopy of blood smear		U

6.5.2.3 Second Line Detection ‘In the Lab’

Table 6-54: Second Line Detection *Bacillus Anthracis*.

Test Method	Sample	Comment	Reference/Company	Code
Culture		Columbia blood agar, aerobe, 12 – 45 °C, opt. 35°C		U
Microscopy		Direct microscopy, Capsule stain Foth, Gram stain, G+, large rod, 1 – 1.5 x 4 – 10 µm, short chains, spore forming (highly stable), capsule forming, non motile		U
PCR			Qiagen https://www.qiagen.com/de/shop/genes-and-pathways/microbial-details.aspx?bacinfoid=42 BioFire http://biofiredefense.com/razorex/razor-ex-pouches/	N/A

6.5.3 *Bartonellae*

Biosafety Level 2.

6.5.3.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/bartonella-bacilliformis-eng.php>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/bartonella-quintana-eng.php>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/bartonella-henselae-eng.php>.

6.5.3.2 First Line Detection ‘In the Field’

Table 6-55: First Line Detection *Bartonellae*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Blood, tissue lesions	Direct microscopy on blood smears, low sensitivity		L

6.5.3.3 Second Line Detection ‘In the Lab’

Table 6-56: Second Line Detection *Bartonellae*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Blood, serum, cardiovascular biopsy, skin lesions	Columbia blood agar, Chocolate agar, Microaerophil, 7 – 14 days until 3 months, 37°C in 5% CO ₂ Cell culture: Vero, HeLa, L929 etc. cell lines at 37°C		L
Microscopy	Blood	Giemsa stain or Diff Quick is better than Gram stain Gimenez stain, IF microscopy, immunohistochemical staining of affected tissues G-, small rod		L
Western blotting				L
IF	Blood	Detection of specific antibodies		L

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
MALDI-TOF	Isolated bacterial cultures	Only differentiation of isolates		L
Molecular biology methods: PCR, real-time PCR, sequencing	Blood, serum, affected tissues (heart valves, skin lesions)	Preferentially: Firstly screening with <i>Bartonella</i> genus-specific real-time PCR (based on internal transcribed spacer, <i>gltA</i> or <i>rpoB</i>); then species-specific qPCR (<i>B. quintana</i> -specific, <i>B. henselae</i> -specific etc.)	Qiagen Microbial DNA qPCR Multi-Assay Kit	L

6.5.4 Lyme Disease *Borrelia*

Biosafety Level 2.

6.5.4.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds21e-eng.php>.

6.5.4.2 First Line Detection ‘In the Field’

Table 6-57: First Line Detection Lyme Disease *Borrelia*.

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay	Liquor, blood	Ab detection	Bionote http://www.bionote.co.kr/File/Upl oad/2015/04/17/2015-04-17(2).pdf	R

6.5.4.3 Second Line Detection ‘In the Lab’

Table 6-58: Second Line Detection Lyme Disease *Borrelia*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Cutis, liquor, blood, joints, synovial fluid	Barbour-Stoenner-Kelly liquid medium (BSK-H), Difficult, 7 – 14 days, 30 – 37 °C, microaerophil Experienced personnel needed		U

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Cutis, liquor, blood, joints	Darkfield, Giemsa, Acridin-orange, G-, spiral shape, motile IF and immunochemical staining in skin biopsies		U
IF	Serum	Ab, standard screening method	e.g. Fuller http://www.antibody-antibodies.com/product4231298-Fuller-Borrelia_burgdorferi_IgG_IFA_Kit_Human.html Progen http://www.progen.de/en/borrelia-antibody-ifa.html Megacor (dog) http://www.megacor.at/product/megafluor_borrelia_canis.html	U
ELISA	Serum	Ab, Many false-positive and false-negative results	IDEXX (dog) https://www.idexx.com/equine/reference-laboratories/testmenu/tests/lyme-anaplasma.html Mikrogen (dog) http://www.mikrogen.de/english/deutschland/products/product-overview/testsystem/borrelia-igg-2.html	CE
Western blotting	Serum	For Western blot follow the CCDC criteria: an IgM immunoblot be considered positive if two of the following three bands are present: 24 kDa (OspC), 39 kDa (BmpA), and 41 kDa (Fla); IgG immunoblot to be considered positive if five of the following 10 bands are present: 18 kDa, 21 kDa (OspC), 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa (Fla), 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa		CE

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
Molecular biology methods: PCR, real-time PCR, sequencing	Serum, blood, liquor, synovial fluid, pericardial fluid, skin biopsies	Preferentially: Firstly screening with <i>Borrelia</i> genus-specific real-time PCR (based on 16S rRNA gene); then species-specific qPCR (<i>B. burgdorferi</i>-specific, <i>B. afzelii</i>-specific, etc.) Amplification and sequencing of a portion of gene (ospA, ospC, flagellin, etc.) for exact species identification in case of difficulties	Qiagen-Artus https://www.qiagen.com/de/shop/detection-solutions/human-pathogens/artus-borrelia-lc-pcr-kit-ce#orderinginformation	L, U

6.5.5 *Brucellae*

Biosafety Level 2 (clinical material during screening).

Biosafety Level 3 (positive screened material, cultures, animal studies).

6.5.5.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds23e-eng.php>.

6.5.5.2 First Line Detection 'In the Field'

Table 6-59: First Line Detection *Brucellae*.

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay	Blood, liquor, synovia, purulence	Ab detection	Tetracore http://www.tetracore.com/bio-warfare/	R
Microscopy		Direct microscopy of clinical material from animals		U

6.5.5.3 Second Line Detection ‘In the Lab’

Table 6-60: Second Line Detection *Brucellae*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Blood, liquor, synovia, purulence	Columbia blood agar, Gassner agar, MacConkey 2 – 3 days (up to 7 days), 5 – 10 % CO ₂ , strict aerobe, G-, very small coccobacilli, non motile		U
Microscopy		<i>Brucella</i> stains (Köster, Hansen, Stamp, Stableforth), GRAM stain		U
CFT/Rose Bengal Test		Lack sensitivity, may be useful in high endemic regions because of high specificity		U
IFT			<i>B. canis</i> (Megacor, dog) http://www.megacor.at/product.html?language=en	U
ELISA	Serum		Virotech http://www.sekisuidiagnostics.com/products/438-brucella-igg-igm-elisa-virotech Novatec http://www.novatec-id.com/de/produkte/novalisa/bakteriologie/brucella/ PrioCHECK (ruminants) https://www.thermofisher.com/order/catalog/product/7610700	CE, U
PCR			BioFire http://biofiredefense.com/reagents/	L

6.5.6 *Burkholderia Mallei* / *Pseudomallei*

Biosafety Level 3.

6.5.6.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds25e-eng.php>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds26e-eng.php>.

DETECTION METHODS

6.5.6.2 First Line Detection ‘In the Field’

Table 6-61: First Line Detection *Burkholderia Mallei* / *Pseudomallei*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy		Direct microscopy		U

6.5.6.3 Second Line Detection ‘In the Lab’

Table 6-62: Second Line Detection *Burkholderia Mallei* / *Pseudomallei*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Sputum, urine, liquor, blood, purulence	Mac Conkey, Columbia blood agar, Ashdown Medium, aerobe, G-, rod, non-motile Long incubation time		U
Microscopy		GRAM stain		U
MALDI-TOF		Only for isolates		U

6.5.7 *Campylobacter*

Biosafety Level 2.

6.5.7.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/campylobacter-coli-eng.php>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/campylobacter-jejuni-eng.php>.

6.5.7.2 First Line Detection ‘In the Field’

Table 6-63: First Line Detection *Campylobacter*.

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay	Faeces, swab	Low sensitivity	Merck millipore http://www.merckmillipore.com/INTL/en/products/industrial-microbiology/pathogen-and-spoilage-testing/pathogen-detection/pathogen-detection-by-product/lateral-flow-tests/mU6b.qB.VfkAAAF_A2_xkiQpx,nav	R

6.5.7.3 Second Line Detection ‘In the Lab’

Table 6-64: Second Line Detection *Campylobacter*.

Test Method	Sample	Comment	Reference/Company	Code
Culture		Karmali agar, Butzler agar, Skirrow agar, Bolton broth, 1 – 3 days, 42 – 43 °C, 5 – 20 % CO ₂ , microaerophil, transport in Amies / Stuart medium G-, tin curved rod (comma shaped), polar flagellata, motile		D
MALDI-TOF		Only for isolates		U
PCR	Food, animal feed, and pharmaceutical products		Congen http://www.congen.de/produkte-services/lebensmittelanalytik/mikroorganismen/ Mericon https://www.qiagen.com/de/shop/detection-solutions/food-safety/mericon-campylobacter-triple-kit#orderinginformation BioFire http://biofiredefense.com/reagents/	U

6.5.8 *Chlamydomophila Psittaci*

Biosafety Level 2 (patient diagnostics during screening).

Biosafety Level 3 (infectious or potentially infectious materials, animals, cultures).

6.5.8.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/chlamydomophila-psittaci-eng.php>.

6.5.8.2 First Line Detection ‘In the Field’

Table 6-65: First Line Detection *Chlamydomophila Psittaci*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Fetus, material from abort	Low sensitivity		R

DETECTION METHODS

6.5.8.3 Second Line Detection ‘In the Lab’

Table 6-66: Second Line Detection *Chlamydomydia Psittaci*.

Test Method	Sample	Comment	Reference/Company	Code
Cell culture		McCoy		L
IF		Ab, is not stained with Lugol solution, round elementary corpuscle		L
CFT		Lack sensitivity, may be useful in high endemic regions because of high specificity		L
ELISA			IDEXX (Chlam. abortus) https://www.idexx.com/livestock-poultry/ruminant/chlamydia.html	U
PCR			LSI VetMAX™ <i>Chlamydomydia spp.</i> Real-Time PCR Kit	R/U

6.5.9 *Clostridium Botulinum*

Biosafety Level 2.

6.5.9.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/clostridium-eng.php>.

6.5.9.2 First Line Detection ‘In the Field’

Table 6-67: First Line Detection *Clostridium Botulinum*.

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay		Only for the detection of the toxin, only confirmatory in case of a positive result	Tetracore http://tetracore.com/elisa-kits/index.html Miprolab http://www.miprolab.com/55.html?&L=1	R

6.5.9.3 Second Line Detection ‘In the Lab’

Table 6-68: Second Line Detection *Clostridium Botulinum*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Liver, serum/blood, swab, stool, vomit and gastric aspirates; environmental samples (soil, surface water), food products	Columbia blood, Zeissler Agar, Chopped meat Anaerobic, β -haemolysis (not all strains)		U
Microscopy		Gram stain, Spore stain Rakette, G+, large rod, 1 x 10 (or more) μ m, spore forming, motile		U
ELISA		Toxin detection	Tetracore http://tetracore.com/elisa-kits/index.html	R
Mouse Bioassay		Experienced personnel needed		nk
PCR			BioFire http://time.idahotech.com/FilmArray/index-BioDef.html	U

6.5.10 *Coxiella Burnetii*

Biosafety Level 3.

6.5.10.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/coxiella-burnetii-eng.php>.

6.5.10.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

DETECTION METHODS

6.5.10.3 Second Line Detection 'In the Lab'

Table 6-69: Second Line Detection *Coxiella Burnetii*.

Test Method	Sample	Comment	Reference/Company	Code
Culture		Cell culture (McCoy), difficult		U
Microscopy	Serum/blood, urine, faeces, milk, placenta, tissue, sputum	Stamp stain, Gimenez stain G-, small rod, 0.3 x 1 µm		U
ELISA			IDEXX (ruminants) https://www.idexx.com/livestock-poultry/ruminant/q-fever.html Virion Serion http://www.virion-serion.de/en/products/serion-elisa-classic-kursiv/respiratory-infectious-diseases/coxiella-burnetii.html	U
IF	Serum, plasma	IgG IgM phase-specific	Fuller http://www.fullerlabs.com/files/8713/0877/8390/QG-120-English.pdf Virotech http://www.sekisuidiagnostics.com/products/459-q-fever-ifa-igg-focus	U
Complement fixation test		Lack sensitivity, may be useful in high endemic regions because of high specificity		L
PCR			Adiavet-BioMerieux http://www.biomerieux-industry.com/veterinary-diagnostics/adiavet-real-time-PCR-diagnostic-kits-for-bovine-species LSI http://www.thermofisher.com/search/results?query=COXIELLA&resultPage=1&resultsPerPage=15&autocomplete=	U

6.5.11 *Erlichia*

Biosafety Level 2.

6.5.11.1 PSDS

See Annex E.

6.5.11.2 First Line Detection ‘In the Field’

Table 6-70: First Line Detection *Erlichia*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Blood, liquor	Giemsa of Diff Quick, better than Gram stain		
Lateral Flow Assay	Blood, liquor	Ab dog	Bionote http://www.bionote.co.kr/animal/eng/production/canine.asp?LT=2&MODE=V&bIdx=515&bcIdx=9&P=1&BLT=L&PC=20&gAIdx=&gSearchText= IDEXX https://www.idexx.com/small-animal-health/products-and-services/snap-4dx-plus-test.html	R

6.5.11.3 Second Line Detection ‘In the Lab’

Table 6-71: Second Line Detection *Erlichia*.

Test Method	Sample	Comment	Reference/Company	Code
Culture		Cell culture, DH82, difficult Experienced personnel needed		R
Microscopy	Serum/blood, urine, faeces, milk, placenta, tissue, sputum	Giemsa stain, Gram stain, Diff-Quick of blood smear G-, small, pleomorphic		U
ELISA	Serum	Ab dog	Immunocomb Biogal http://www.biogal.co.il/products/immunocomb/the-unique-advanta-ges-for-specific-immunocomb-kits/the-immunocomb-canine-ehrlichia-kit	R

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
IF			Megacor (dog) http://www.megacor.at/product.html?language=en	U
PCR			Gerbion http://www.gerbion.de/pdf/G01033.pdf	L

6.5.12 *Escherichia Coli* Enteropathogenic

Biosafety Level 2.

6.5.12.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/escherichia-coli-pa-eng.php>.

6.5.12.2 First Line Detection ‘In the Field’

Table 6-72: First Line Detection *Escherichia Coli* (Enteropathogenic).

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay	Faeces	For shigatoxin 1 and 2 (EHEC) low sensitivity without enrichment		R

6.5.12.3 Second Line Detection ‘In the Lab’

Table 6-73: Second Line Detection *Escherichia Coli* (Enteropathogenic).

Test Method	Sample	Comment	Reference/Company	Code
Culture	Faeces, blood, tissue, swab	Mac Conkey, Gassner, Chromagar, Columbia Blood Agar, Gassner Agar 12 – 48 °C, opt. 37 – 42 °C, oxidase negative, katalase positive, indol positive		U
Microscopy		G-, rod, 2 – 6 x 1.1 – 1.5 µm, flagellata, motile		U
MALDI-TOF		Only for isolates		U
PCR	Stool, culture		Congen http://www.r-biopharm.com/items/ridagene-ehecepec	

Test Method	Sample	Comment	Reference/Company	Code
PCR (cont'd)			Mericon https://www.qiagen.com/de/shop/genes-and-pathways/pathway-details.aspx?pwid=152 R-Biopharm http://www.r-biopharm.com/wp-content/uploads/Pathogenic_E-coli-STECEHEC-EPEC-detection-test-kits.pdf	U

6.5.13 *Francisella Tularensis*

Biosafety Level 2 (clinical material).

Biosafety Level 3 (cultures, animal studies).

6.5.13.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds68e-eng.php>.

6.5.13.2 First Line Detection 'In the Field'

Table 6-74: First Line Detection *Francisella Tularensis*.

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay			Tetracore http://www.tetracore.com/bio-warfare/index.html Miprolab http://www.miprolab.com/fileadmin/Dateiablage/PDF/MF512-001-02_PriceList-miPROTECT_Testsystem.pdf	R

DETECTION METHODS

6.5.13.3 Second Line Detection ‘In the Lab’

Table 6-75: Second Line Detection *Francisella Tularensis*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Lesion exudate, respiratory secretion, cerebrospinal fluid	Heart-cystein-blood-agar Fastidious, strict aerobe, 3 – 7 days		U
Microscopy		Giemsa stain is better than Gram stain, G-, very small tin rod (coccobacilli), maximum length 0.7 µm, capsule forming, non motile		U
ELISA	Serum/blood	IgG, IgM	Virion/serion http://www.virion-serion.de/download/flyer/Flyer_ELISA_classic_Francisella_tularensis_EN.pdf	U
IF			LSBio https://www.lsbio.com/antibodies/anti-francisella-tularensis-lps-antibody-mouse-anti-francisella-tularensis-monoclonal-elisa-if-immunofluorescence-ls-c102914/104645	R
PCR			BioFire http://biofiredefense.com/pdfs/FilmArray/Infosheet,%20FilmArray%20Biothreat-0039.pdf	U

6.5.14 *Leptospira Interrogans*

Biosafety Level 2.

6.5.14.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds95e-eng.php>.

6.5.14.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.5.14.3 Second Line Detection ‘In the Lab’

Table 6-76: Second Line Detection *Leptospira Interrogans*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Blood, serum, liquor, urine	Liquid <i>Leptospira</i> medium Obligatory aerobe, 28 – 32 °C, 1 – 2 weeks (up to 26 weeks)		U
Microscopy	Blood, serum, liquor, urine	Spiral shaped, Darkfield Typical spiral shape with hooky ends, rod, flagellata, motile		U
ELISA	Serum, plasma	IgG, IgM	Novatec http://www.novatec-id.com/de/produkte/novalisa/bakteriologie/leptospira/	L
MAT		Reference method, experienced personnel needed		U
PCR			Adiavet-BioMerieux http://www.biomerieux-industry.com/veterinary-diagnostics/lepto-leptospira QIAGEN https://www.qiagen.com/de/shop/pcr/primer-sets/microbial-dna-qpcr-assay-kits?catno=BBID00203A#orderinginformation	L

6.5.15 *Listeria Monocytogenes*

Biosafety Level 2.

6.5.15.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/listeria-monocytogenes-eng.php>.

6.5.15.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

DETECTION METHODS

6.5.15.3 Second Line Detection ‘In the Lab’

Table 6-77: Second Line Detection *Listeria Monocytogenes*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Blood, liquor, tissue, food products	Palcam agar, Oxford agar, Fraser-Broth, Blood agar Non fastidious, facultative anaerobe, 4 – 37 °C,		D
Microscopy		G+ (G labile), short rod, length 0.5 – 2 µm, lagellate, motile (up to 25°C)		U
MALDI-TOF		Only for isolates		U
IFT				nk
PCR			Congen http://www.congen.de/en/produktueckruefe-listeria-monocytogenes/ Mericon https://www.qiagen.com/de/shop/detection-solutions/food-safety/mericon-l-monocytogenes-kit#orderinginformation BioFire http://biofiredefense.com/reagents/	U

6.5.16 *Mycobacterium Bovis*

Biosafety Level 3.

6.5.16.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/mycobacterium-eng.php>.

6.5.16.2 First Line Detection ‘In the Field’

Table 6-78: First Line Detection *Mycobacterium Bovis*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy		Direct microscopy on smears (Ziehl Neelsen stain)	KIT QUICK TB (RAL) / ELVETEC (217767)	

6.5.16.3 Second Line Detection ‘In the Lab’

Table 6-79: Second Line Detection *Mycobacterium Bovis*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Sputum, tissue	Löwenstein-Jensen medium, difficult Microaerophil, 3 – 6 weeks, 37°C When cultivated on solid medium, <i>M. bovis</i> was grown on Middlebrook 7H10 agar supplemented with (OADC) for 3 weeks at 37°C	SIGMA (63237-500G-F) MIDDLEBROOK 7H10 AGAR/ BECTON DICKINSON (262710) MIDDLEBROOK OADC/ BECTON DICKINSON (211886)	nk
Microscopy		Ziehl-Neelsen, Gram stain is impossible Acid resistant, very tin, rod, sometimes lightly curved, 0.3 – 0.6 x 1 – 4 µm, non motile	KIT QUICK TB (RAL) /ELVETEC (217767)	R
MALDI-TOF		Only for isolates	Bruker	L
PCR	Sputum, tissue		Gene Proof http://www.geneproof.com/products/human-microbiological-diagnostics/mycobacterium-tuberculosis-pcr-kit Norgen, QIAGEN	R

6.5.17 *Pasteurella Multocida*

Biosafety Level 2.

6.5.17.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/pasteurella-spp-eng.php>.

6.5.17.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

DETECTION METHODS

6.5.17.3 Second Line Detection ‘In the Lab’

Table 6-80: Second Line Detection *Pasteurella Multocida*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Skin biopsy, rare liquor, endocard, neonates, swab	Columbia blood agar, Chocolate agar Facultative anaerobe, 5 – 7 % CO ₂ , 25 – 40 °C, opt. 37°C		R
Microscopy		Methylenblue stain, Giemsa stain G-, small, pleomorphic, coccobacilli, markedly polar (Giemsa stain increase polarity), maximum length 1 µm, non motile		U
ELISA		ELISA (toxin detection)	Thermo Scientific http://www.oxid.com/UK/blue/rod_detail/rod_detail.asp?pr=K000921&c=UK&lang=EN DAKO http://www.analisisavanzados.com/modules/mod_tecdata/K000911-9_PM_Toxin_ELISA_Kit.pdf	L
MALDI-TOF		Only for isolates		L

6.5.18 *Rickettsiae*

Biosafety Level 2/3.

6.5.18.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/rickettsia-akari-eng.php>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/rickettsia-prowazekii-eng.php>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/rickettsia-rickettsii-eng.php>.

6.5.18.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.5.18.3 Second Line Detection ‘In the Lab’

Table 6-81: Second Line Detection *Rickettsiae*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Blood, serum, skin biopsy, skin swab	Cell culture L929, XTC-2, Vero cell lines Requires experienced laboratory staff		U
Microscopy		Gimenez stain G-, small coccobacilli, 0.2 – 0.5 µm Native material (skin biopsy): immunohistochemical staining; IF		U
IF	Serum, blood	Standard screening is recommended with at least several rickettsial antigens	Fuller http://www.fullerlabs.com/products/veterinary/usa_and_canada/dv-rickettsia/dv-rickettsia_ifa/ Virotech http://www.sekisuidiagnostics.com/products/612-rickettsia-ifa-igm-focus	U
ELISA	Serum	Not widely used	Fullerlabs http://www.fullerlabs.com/index.php/products/veterinary/usa_and_canada/dv-rickettsia/dv-rickettsia_elisa	L
Western blotting		Many cross-reactions among spotted fever group <i>Rickettsiae</i> . WB and cross-absorption may help in identification of the causative agent		L

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
Molecular biology methods: PCR, real-time PCR, sequencing	Blood, skin biopsies, swabs from skin lesions (eschars)	Preferentially: Firstly screening with <i>Rickettsia</i> genus-specific real-time PCR (based on <i>gltA</i> or 16S rRNA gene); then species-specific qPCR (<i>R. prowazekii</i>-specific, <i>R. conorii</i>-specific, <i>R. rickettsii</i>-specific etc.) Amplification and sequencing of a portion of gene (<i>gltA</i>, <i>ompA</i>, etc.) for exact species identification in case of difficulties		U

6.5.19 *Salmonellae*

Biosafety Level 2/3.

6.5.19.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/salmonella-ent-eng.php>.

6.5.19.2 First Line Detection ‘In the Field’

Table 6-82: First Line Detection *Salmonellae*.

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay	Culture		BIORAD http://www.bio-rad.com/en-ch/sku/3556710-salmonella-latex-test MERCK-MILLIPORE https://www.merckmillipore.com/INTL/en/product/Singlepath-Salmonella,MDA_CHEM-104140 3M TECRA http://solutions.3m.com/wps/portal/3M/en_WW/Food_Safety/home/Products/one/?PC_Z7_RJH9U52308M770IC4G5E58108000000_nid=4PNR12ZMLDbeD5SVNVK79Cgl	R

6.5.19.3 Second Line Detection ‘In the Lab’

Table 6-83: Second Line Detection *Salmonellae*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Faeces, blood, tissue, food, water	MacConkey agar, Rambach agar, Gassner agar, XLD agar, Chromagar Ramabaquick, Chromagar <i>Salmonella</i> , <i>Salmonella</i> plus facultative anaerobe, 37°C, oxidase negative, katalase positive, indol negative, urease negative		D
Microscopy		G-, rod, 2 µm, flagellata, motile		U
ELISA		Ab (pig)		L
MALDI-TOF	Culture	Only for isolates		U
PCR			Congen http://www.congen.de/en/salmonellenachweis-innerhalb-von-18-26-h/ Mericon https://www.qiagen.com/de/shop/detection-solutions/food-safety/mericon-salmonella-spp-kit#orderinginformation BioFire http://biofiredefense.com/razorex/razor-ex-pouches/	U

6.5.20 *Spirillum Minus*

Biosafety Level 2.

6.5.20.1 PSDS

<http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.180.5295&rep=rep1&type=pdf>.

6.5.20.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

DETECTION METHODS

6.5.20.3 Second Line Detection ‘In the Lab’

Table 6-84: Second Line Detection *Spirillum Minus*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy		Darkfield, Giemsa G-, spiral shaped rod, maximum 3 coils, length 3 – 5 µm, motile		L

6.5.21 *Streptobacillus Moniliformis*

Biosafety Level 2.

6.5.21.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/streptobacillus-eng.php>.

6.5.21.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.5.21.3 Second Line Detection ‘In the Lab’

Table 6-85: Second Line Detection *Streptobacillus Moniliformis*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Skin biopsy, blood, synovia, body fluids	Spec media Difficult, fastidious, aerobe, higher CO ₂ concentration (no strict anaerobe), 37°C		L
Microscopy		Darkfield, Giemsa G-, pleomorphic, short rod, curved, sometimes filamented, maximum width 1 µm, length 1 – 5 µm (sometimes 10 – 100 µm)		L

6.5.22 *Streptococcus Suis*

Biosafety Level 2.

6.5.22.1 PSDS

See Annex F.

6.5.22.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.5.22.3 Second Line Detection ‘In the Lab’

Table 6-86: Second Line Detection *Streptococcus Suis*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Blood, liquor, tissue	Blood agar Facultative anaerobe, 10 – 45 °C, opt. 37°C, α-haemolysis		U
Microscopy		G+, coccus, two or short chains		U
MALDI-TOF	Culture			L

6.5.23 *Yersinia Enterocolitica*

Biosafety Level 2.

6.5.23.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/yersinia-enterocolitica-eng.php>.

6.5.23.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.5.23.3 Second Line Detection ‘In the Lab’

Table 6-87: Second Line Detection *Yersinia Enterocolitica*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Faeces, blood, liquor, aspirate	Columbia Blood Agar, Gassner Agar, CIN agar (lacks specificity) Facultative anaerobe, 25 – 37 °C		U
Microscopy		Methylenblue stain, Giemsa G-, markedly polar (Giemsa stain increase polarity), rod or coccobacilli, 0.4 – 0.8 x 1 – 3 µm, flagellata, motile (22 – 30 °C)		U

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
ELISA	Serum, plasma, meat juice		Virion-Serion http://www.virion-serion.de/download/flyer/Flyer_ELISA_classic_Yersinia_EN.pdf Virotech http://www.sekisuidiagnostics.com/products/642-yersinia-enterocolitica-igg-iga-elisa-virotech QIAGEN (pig) https://www.qiagen.com/de/shop/detection-solutions/animal-pathogens/pigtype-yersinia-ab#orderinginformation Tecan http://www.ibl-international.com/en/yersinia-enterocolitica-igg	L
IFT	Serum		Viramed http://www.viramed.de/en/bacteria/yersinia-species/yersinia-virastripe/13-produkte/55-yersinia-virastripe-igg-iga-test-kit Biosite http://www.bios-world.com/e/gi-lang.php	L
MALDI-TOF		Only for isolates		U
PCR	Food, animal feed, and pharmaceutical products		Mericon https://www.qiagen.com/de/shop/detection-solutions/food-safety/mericon-y-enterocolitica-kit#orderinginformation	L

6.5.24 *Yersinia Pestis*

Biosafety Level 3.

6.5.24.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds169e-eng.php>.

6.5.24.2 First Line Detection ‘In the Field’

Table 6-88: First Line Detection *Yersinia Pestis*.

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay			Tetracore http://www.tetracore.com/molecular-biothreat/index.html Miprolab http://www.miprolab.com/55.html?&L=1 Abicap http://www.diavita.de/germanneue/images/stories/Downloads/ProduktPreisliste_2008.pdf	R
Microscopy		Direct microscopy of blood smear		U

6.5.24.3 Second Line Detection ‘In the Lab’

Table 6-89: Second Line Detection *Yersinia Pestis*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Blood, liquor, aspirate	Columbia Blood Agar, Gassner Agar, CIN agar (lacks specificity) Non fastidious, facultative anaerobe, 0 – 40 °C, opt. 30°C		U
Microscopy		Methyleneblue stain, Giemsa G-, 0.5 – 0.8 x 1 – 3 µm, markedly polar (Giemsa stain increase polarity), rod (coccobacilli up to filamented), non motile		U
MALDI-TOF	Culture	Only for isolates with spec. database		U
ELISA		F1-Antigen		U
PCR			BioFire http://biofiredefense.com/reagents/	U

DETECTION METHODS

6.5.25 *Yersinia Pseudotuberculosis*

Biosafety Level 2.

6.5.25.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/yersinia-pseudotuberculosis-eng.php>.

6.5.25.2 First Line Detection ‘In the Field’

There are currently no first line detection methods available.

6.5.25.3 Second Line Detection ‘In the Lab’

Table 6-90: Second Line Detection *Yersinia Pseudotuberculosis*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Faeces, blood, liquor, aspirate, sputum	Columbia Blood Agar, Gassner Agar, CIN agar (lacks specificity) Facultative anaerobe, 25 – 30 °C		U
Microscopy		Methylenblue stain, Giemsa G-, markedly polar (Giemsa stain increase polarity), rod, motile (22 – 30 °C)		U
ELISA		F1-Antigen		L
MALDI-TOF		Only for isolates		U

6.5.26 *Staphylococcus Aureus*

Biosafety Level 2.

6.5.26.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/staphylococcus-aureus-eng.php>.

6.5.26.2 First Line Detection ‘In the Field’

Table 6-91: First Line Detection *Staphylococcus Aureus*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy		Direct microscopy for wound infections is possible but lacks sensitivity and specificity		U
Lateral Flow Assay		Enterotoxin B		U

6.5.26.3 Second Line Detection ‘In the Lab’

Table 6-92: Second Line Detection *Staphylococcus Aureus*.

Test Method	Sample	Comment	Reference/Company	Code
Culture	Blood, tissue, purulence, swab	Blood agar, BairdParker agar, MRSA agar, Chromagar – <i>S. aureus</i> Pastorex (BioRad) (Plasma coagulase reaction takes 24 hrs) Facultative anaerobe, 7 – 46 °C, opt. 30°C, plasmacoagulase positive, pigmented, β-haemolysis		D
Microscopy		Gram stain, G+, coccus, 0.5 – 1.5 µm, clusters, some strains thick capsule, non motile		U
MALDI-TOF		Only for isolates, protocols published for detection of MRSA in blood culture		U
ELISA	Food, culture, fluid	Enterotoxin A-E	R-Biopharm http://www.r-biopharm.com/products/food-feed-analysis/microbiology-hygiene/staphylococcal-enterotoxin-set/item/ridascreen-set-total	U

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
ELISA (cont'd)			Transia http://www.biocontrolsys.com/products/view/TPSE	
PCR			Congen http://www.congen.de/en/search-products/?service=&detection=&analyte=staphylococcus-aureus-2	L

6.5.27 Relapsing Fever *Borrelia*

Biosafety Level 2.

6.5.27.1 PSDS

<http://www.cdc.gov/relapsing-fever/>.

<http://www.aafp.org/afp/2005/1115/p2039.html>.

6.5.27.2 First Line Detection 'In the Field'

Table 6-93: First Line Detection Relapsing Fever *Borrelia*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Blood	Giemsa-stained thick and thin blood smears observation may provide rapid identification in case of acute infection		L

6.5.27.3 Second Line Detection 'In the Lab'

Table 6-94: Second Line Detection Relapsing Fever *Borrelia*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Blood, liquor	Giemsa-stained thick and thin blood smears Darkfield G-, large, spiral shaped, 5 – 10 coils, length 30 µm, motile Peripheral blood smears of a symptomatic/febrile person		L

Test Method	Sample	Comment	Reference/Company	Code
Culture	Blood before antibiotic treatment	Barbour Stoner Kelly Medium, 24 h		L
ELISA		May be used for the screening purposes, antibodies has low specificity and rapidly disappears		L
IF		Ab – not standardized		L
Molecular biology methods: PCR, real-time PCR, sequencing	Blood, serum, liquor	Preferentially: Firstly screening with <i>Borrelia</i> genus-specific real-time PCR (based on 16S rRNA gene); then species-specific qPCR (<i>B. recurrentis</i>-specific, <i>B. duttonii</i>-specific, <i>B. crocidurae</i>-specific, etc.) Amplification and sequencing of a portion of gene (<i>flaB</i>, <i>glpQ</i>, etc.) for exact species identification in case of difficulties		L

6.6 DETECTION METHODS OF PARASITES

6.6.1 *Angiostrongylus Cantonensis*

Biosafety Level 2.

6.6.1.1 PSDS

<http://www.cdc.gov/parasites/angiostrongylus/>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/acantonensis-eng.php>.

6.6.1.2 First Line Detection ‘In the Field’

Detection methods are based on cerebrospinal fluid samples. No first line detection methods exist.

DETECTION METHODS

6.6.1.3 Second Line Detection ‘In the Lab’

Table 6-95: Second Line Detection *Angiostrongylus Cantonensis*.

Test Method	Sample	Comment	Reference/Company	Code
ELISA	50 – 100 µl serum or plasma 100 mg tissue sample	Cross-reaction may exist for other species.	Anti- <i>Angiostrongylus cantonensis</i> Antibody (IgG and IgM) ELISA Kit, antibodies-online http://www.antibodies-online.com/kit/832296/Anti-Angiostrongylus+Cantonensis+IgG+ELISA/	R

6.6.2 *Ascaris Lumbricoides*

Biosafety Level 2.

6.6.2.1 PSDS

<http://www.cdc.gov/parasites/ascariasis/>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds10e-eng.php>.

6.6.2.2 First Line Detection ‘In the Field’

Gold standard is the diagnosis made by identifying worms or eggs in feces. Sensitivity is high due to the large quantity of eggs laid.

Table 6-96: First Line Detection *Ascaris Lumbricoides*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stool	Detection of ova and parasites	http://www.cdc.gov/dpdx/ascariasis/gallery.html	N/A
Microscopy	Sputum	Larvae may be identified during pulmonary migration Cave: same with interfering larvae of geohelminths		
Macroscopic	Adult ♀ worms in feces or migration through nose or mouth			

6.6.2.3 Second Line Detection ‘In the Lab’

Table 6-97: Second Line Detection *Ascaris Lumbricoides*.

Test Method	Sample	Comment	Reference/Company	Code
ELISA	50 – 100 µl serum or plasma 100 mg tissue sample	Cross-reaction may exist for other species	http://www.antibodies-online.com/kit/832298/Anti-Ascaris+Lumbricoides+IgM+ELISA	R
RT-PCR	Extracted DNA from stools	Easy, Standard and Advance kits exist; for Easy kit the genesig q16 instrument is needed!	<i>Ascaris lumbricoides</i> / <i>Ascaris suum</i> Easy Kit, Standard Kit, Advanced Kit, genesig, http://www.genesig.com/assets/files/a_1_lumbricoides.pdf	R

6.6.3 *Babesia Microti*, *Babesia Divergens*

Biosafety Level 2.

6.6.3.1 PSDS

<http://www.cdc.gov/parasites/babesiosis/>.

6.6.3.2 First Line Detection ‘In the Field’

http://www.cdc.gov/dpdx/resources/pdf/benchAids/Babesia_benchaid.pdf.

Table 6-98: First Line Detection *Babesia Microti*, *Babesia Divergens*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	EDTA blood (min 2 ml) Capillary blood	Thick and thin blood films stained with Giemsa or other appropriate stains (Diff quick) Repeated smears may be necessary in order to increase sensitivity	http://www.cdc.gov/dpdx/babesiosis/gallery.html	N/A

DETECTION METHODS

6.6.3.3 Second Line Detection ‘In the Lab’

Table 6-99: Second Line Detection *Babesia Microti*, *Babesia Divergens*.

Test Method	Sample	Comment	Reference/Company	Code
IFA	Serum (1 ml)	<i>B. microti</i> only <i>Babesia</i> IgG detectable control only	<i>Babesia (B. microti)</i> IFA Substrate Slide Focus Diagnostics, Cypress, USA http://www.focusdx.com/product-catalog/if0904-babesia-b-microti-ifa-substrate-slide-ous/ https://www.focusdx.com/product/IF0910/ous	
RT-PCR	0,7 ml whole blood (ACD or EDTA) or Tick (in EtOH or alive)	<i>B. microti</i> only	<i>Babesia microti</i> DNA Real-Time PCR Focus Diagnostics, Cypress, USA	

6.6.4 *Balantidium Coli*

Biosafety Level 2.

6.6.4.1 PSDS

<http://www.cdc.gov/parasites/balantidium/>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds15e-eng.php>.

6.6.4.2 First Line Detection ‘In the Field’

Table 6-100: First Line Detection *Balantidium Coli*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stool	Unstained, trichrome or eosin stained Identification of trophozoites and/or cysts in feces wet smears <i>B. coli</i> is rapidly destroyed outside colon and need immediate examination or preservation <i>B. coli</i> is passed intermittently, which makes repeatedly collected stool specimens necessary in order to increase sensitivity	http://www.cdc.gov/dpdx/balantidiasis/gallery.html	N/A

6.6.4.3 Second Line Detection ‘In the Lab’

Table 6-101: Second Line Detection *Balantidium Coli*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Tissue collected during endoscopy	Unstained, trichrome or eosin stained Identification of trophozoites and/or cysts	http://www.cdc.gov/dpdx/balantidiasis/gallery.html	N/A

6.6.5 *Clonorchis Sinensis (Opisthorchis Sinensis)*

Biosafety Level 2.

6.6.5.1 PSDS

<http://www.cdc.gov/parasites/clonorchis/index.html>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds34e-eng.php>.

6.6.5.2 First Line Detection ‘In the Field’

Table 6-102: First Line Detection *Clonorchis Sinensis (Opisthorchis Sinensis)*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stools	Identification of eggs in faeces	http://www.cdc.gov/dpdx/clonorchiasis/gallery.html	N/A

6.6.5.3 Second Line Detection ‘In the Lab’

Table 6-103: Second Line Detection *Clonorchis Sinensis (Opisthorchis Sinensis)*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Duodenal aspirate	Identification of eggs in faeces and worms from liver ductus	http://www.cdc.gov/dpdx/clonorchiasis/gallery.html	N/A
ELISA	50 – 100 µl serum	IgG only	<i>Clonorchis Sinensis</i> (CS) (IgG) ELISA Kit, antibodies-online http://www.antibodies-online.com/kit/1095070/CS+IgG+ELISA/?utm_source=partner&utm_medium=biocompare&utm_campaign=listing&utm_content=100&utm_term=sponsored	R

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
ELISA	Serum	IgG only	Human <i>Clonorchis Sinensis</i> (CS) antibody, IgG ELISA Kit, MyBioSource http://www.mybiosource.com/images/tds/protocol_others/MS706861.pdf	R

6.6.6 *Cryptosporidium* “Parvum” (*C. hominis*, *C. meleagridis*, *C. felis*, *C. canis*)

Biosafety Level 2.

6.6.6.1 PSDS

<http://www.cdc.gov/parasites/crypto/>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds48e-eng.php>.

6.6.6.2 First Line Detection ‘In The Field’

http://www.cdc.gov/dpdx/resources/pdf/benchAids/Crypto_benchaid.pdf.

Table 6-104: First Line Detection *Cryptosporidium* ‘Parvum’.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stools	Identification of oocysts <i>Cryptosporidium</i> spp. may be detected by routine stool microscopy but with lower sensitivity than with specific stains (modified Kinyoun acid-fast stain) Multiple stool samples should be tested before a negative diagnostic interpretation is reported	http://www.cdc.gov/dpdx/cryptosporidiosis/dx.html	N/A
Lateral Flow Assay	Stool	ImmunoCard STAT!® <i>Crypto/Giardia</i> Results in minutes	http://www.meridianbioscience.com/diagnostic-products/cryptosporidium-and-giardia/immunocard-stat/immunocard-stat-crypto-and-giardia.aspx	D

6.6.6.3 Second Line Detection ‘In the Lab’

Acid-fast staining methods, with or without stool concentration, are most frequently used in clinical laboratories. For greatest sensitivity and specificity, immunofluorescence microscopy is the method of choice (followed closely by enzyme immunoassays). Molecular methods are mainly a research tool for species discrimination.

Table 6-105: Second Line Detection *Cryptosporidium* 'Parvum'.

Test Method	Sample	Comment	Reference/Company	Code
DFA	Stool	Recommended by CDC, more sensitive than traditional staining methods, rapid test (30 minutes) Combination of <i>Cryptosporidium</i> and <i>Giardia</i> detection	Merifluor® <i>Cryptosporidium</i> / <i>Giardia</i> , Meridian Bioscience http://www.meridianbioscience.com/diagnostic-products/cryptosporidium-and-giardia/merifluor/merifluor-cryptosporidium-and-giardia.aspx	D
ELISA rapid test	1 gram stool sample		<i>Cryptosporidium</i> 2 nd Generation (Fecal), Diagnostic Automation/Cortez Diagnostics, Inc., USA (rapidtest.com) http://www.rapidtest.com/index.php?i=Cryptosporidium-Fecal-ELISA-kit&id=185&cat=17	IVD
ELISA	Stool	Combination of <i>Cryptosporidium</i> and <i>Giardia</i> detection	<i>Crypto</i> / <i>Giardia</i> Ag Combo (Fecal) ELISA, Diagnostic Automation/Cortez Diagnostics, Inc., US (rapidtest.com) http://www.rapidtest.com/index.php?i=Crypto/Giardia-Ag-Combo-ELISA-kit&id=622&cat=17	IVD
RT-PCR	Stool (and other types of biological fluids)		<i>Cryptosporidium</i> Test Kit KHCryp for Clinical samples, CeeramTools, France http://www.ceeramtools.com/images/RT-PCR-Kits/Real-Time-PCR-Kits-FT/Cryptosporidium-IVD.pdf	IVD

6.6.7 *Diphyllobothrium Latum*

Biosafety Level 2.

6.6.7.1 PSDS

<http://www.cdc.gov/parasites/diphyllobothrium/index.html>.

6.6.7.2 First Line Detection 'In the Field'

Microscopic identification of eggs in the stool is the basis of specific diagnosis. Eggs are usually numerous and can be demonstrated without concentration techniques. Examination of proglottids passed in the stool is also of diagnostic value.

DETECTION METHODS

Table 6-106: First Line Detection *Diphyllobothrium Latum*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stools	Identification of eggs or segments of the tapeworm More than one stool sample may be needed for safe diagnosis	http://www.cdc.gov/dpdx/diphyllobothriasis/gallery.html	N/A

6.6.7.3 Second Line Detection ‘In the Lab’

Table 6-107: Second Line Detection *Diphyllobothrium Latum*.

Test Method	Sample	Comment	Reference/Company	Code
Multiplex PCR	Stools	Used to differentiate between <i>Diphyllobothrium spp.</i>	Wicht <i>et al.</i> [104] http://www.ncbi.nlm.nih.gov/pubmed/20592146	L

6.6.8 *Echinococcus Granulosus, E. multilocularis*

Biosafety Level 3.

6.6.8.1 PSDS

<http://www.cdc.gov/parasites/echinococcosis/>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds54e-eng.php>.

6.6.8.2 First Line Detection ‘In the Field’

The diagnosis of echinococcosis relies mainly on findings (cysts) by ultrasonography and/or other imaging techniques supported by positive serologic tests.

There are currently no first line laboratory detection methods available.

6.6.8.3 Second Line Detection ‘In the Lab’

EIA or IHA is used to screen all specimens; a positive reaction is confirmed by immunoblot assay or any gel diffusion assay that demonstrates the echinococcal “Arc 5.” (<http://www.cdc.gov/dpdx/echinococcosis/dx.html>).

Table 6-108: Second Line Detection *Echinococcus Granulosus*, *Echinococcus Multilocularis*.

Test Method	Sample	Comment	Reference/Company	Code
ELISA rapid test	Serum (5 µl)	IgG only	AccuDiag™ <i>Echinococcus</i> IgG ELISA Kit, Diagnostic Automation/Cortez Diagnostics, Inc., USA (rapidtest.com) http://www.rapidtest.com/index.php?i=Echinococcus-IgG--ELISA-kit&id=176&cat=17	IVD
IHA	Serum	IgG only	ELI.H.A <i>Echinococcus</i> , ELITech Group, France http://www.elitechgroup.com/corporate/products/market-segment/microbiology/parasitology/eli-h-a-echinococcus-ref295/overview <i>Echinococcus multilocularis</i> , Bordier Affinity Products SA, Switzerland http://www.bordier.ch/9300%20Echinococcus%20multilocularis/index.htm	nk D

6.6.9 Ectoparasites (Sensu Stricto: Lice, Mites, Hard Ticks)

6.6.9.1 PSDS

<http://www.cdc.gov/parasites/lice/index.html>.

<http://www.cdc.gov/parasites/scabies/index.html>.

6.6.9.2 First Line Detection ‘In the Field’

Table 6-109: First Line Detection Ectoparasites.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy, dissecting scope or magnifying glass	Skin scrapes, whole ectoparasites	Direct exam of ectoparasites or skin scrapes (mites) using identification keys	CDC http://www.cdc.gov/dpdx/pedunculosis/gallery.html http://www.cdc.gov/dpdx/scabies/gallery.html	N/A

6.6.9.3 Second Line Detection ‘In the Lab’

See first line detection.

DETECTION METHODS

6.6.10 *Fasciola Hepatica*

Biosafety Level 2.

6.6.10.1 PSDS

<http://www.cdc.gov/parasites/fasciola/>.

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/fasciola-eng.php>.

6.6.10.2 First Line Detection ‘In the Field’

A high index of suspicion is important, especially because the clinical manifestations are nonspecific and the parasitologic tools are suboptimal.

The most widely used diagnostic approach is direct detection of *Fasciola* eggs, by light-microscopic examination of stool or of duodenal or biliary aspirates. However, egg production typically does not start until 3 to 4 months after the exposure, whereas antibodies to the parasite may become detectable 2 to 4 weeks post-infestation. Even during the chronic phase of infection, more than one stool specimen may need to be examined to find the parasite, especially in people with light infections.

Table 6-110: First Line Detection *Fasciola Hepatica*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stool	Identification of eggs, rarely worms	CDC http://www.cdc.gov/dpdx/fascioliasis/gallery.html	N/A

6.6.10.3 Second Line Detection ‘In the Lab’

Table 6-111: Second Line Detection *Fasciola Hepatica*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stools, duodenal or biliary drainage sample	Identification of eggs, rarely worms	CDC http://www.cdc.gov/dpdx/fascioliasis/gallery.html	N/A
ELISA rapid test	5 µl of diluted serum	IgG only	AccuDiag™ <i>Fasciola</i> IgG ELISA Kit, Diagnostic Automation/Cortez Diagnostics, Inc., US (rapidtest.com) http://www.rapidtest.com/pdf/Fasciola%20IgG%208119-35(09-04-2015).pdf	IVD

Test Method	Sample	Comment	Reference/Company	Code
IHA	Serum		ELI.H.A Distoma, ELITech Group, France (TI) http://www.elitechgroup.com/corporate/products/market-segment/microbiology/parasitology/eli-h-a-distoma-ref294/overview	nk

6.6.11 *Giardia Intestinalis* (*G. lamblia*, *G. duodenalis*)

Biosafety Level 2.

6.6.11.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/giardia-lamblia-eng.php>.

<http://www.cdc.gov/parasites/giardia/index.html>.

6.6.11.2 First Line Detection ‘In the Field’

Table 6-112: First Line Detection *Giardia Intestinalis*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	(Unfixed) stool or duodenal aspirate	Direct wet mount or trichrome stain Detection of cysts or falling-leaf motility of trophozoites on direct examination Multiple (min 6) specimens for stool examination may be required (intermittent excretion)	http://www.cdc.gov/dpdx/giardiasis/gallery.html	N/A
Lateral Flow Assay	Stool	ImmunoCard STAT!® <i>Crypto/Giardia</i> Results in minutes	http://www.meridianbioscience.com/diagnostic-products/cryptosporidium-and-giardia/immunocard-stat/immunocard-stat-crypto-and-giardia.aspx	D

6.6.11.3 Second Line Detection ‘In the Lab’

PCR can be used for identification of the subtypes of *Giardia*.

DETECTION METHODS

Table 6-113: Second Line Detection *Giardia Intestinalis*.

Test Method	Sample	Comment	Reference/Company	Code
DFA	Stool	Recommended by CDC, more sensitive than traditional staining methods, rapid test (30 minutes) Combination of <i>Cryptosporidium</i> and <i>Giardia</i> detection	Merifluor® <i>Cryptosporidium/Giardia</i> , Meridian Bioscience http://www.meridianbioscience.com/diagnostic-products/cryptosporidium-and-giardia/merifluor/merifluor-cryptosporidium-and-giardia.aspx	D
ELISA	Stool	Combination of <i>Cryptosporidium</i> and <i>Giardia</i> detection	<i>Crypto/Giardia</i> Ag Combo (Fecal) ELISA, Diagnostic Automation/Cortez Diagnostics, Inc., US (rapidtest.com) http://www.rapidtest.com/index.php?i=Crypto/Giardia-Ag-Combo-ELISA-kit&id=622&cat=17	IVD
Lateral Flow Assay for animal samples	Stool	For animal samples (dogs, cats and livestock)	https://www.idexx.com/small-animal-health/products-and-services/snap-giardia-test.html	D

6.6.12 *Leishmaniae*

Biosafety Level according to the species:

- *Leishmania brasiliensis* 3
- *Leishmania donovani* 3
- *Leishmania ethiopica* 2
- *Leishmania infantum* 2
- *Leishmania major* 2
- *Leishmania mexicana* 2
- *Leishmania peruviana* 2
- *Leishmania tropica* 2
- *Leishmania spp.* 2

6.6.12.1 Primarily Cutaneous Manifestation

6.6.12.1.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/leishmania-eng.php>.

<http://www.cdc.gov/parasites/leishmaniasis/>.

The exact species discrimination of *Leishmania* species outbreaks during deployments is necessary for the correct implementation of adequate preventive countermeasures for proper Force Health Protection.

6.6.12.1.2 First Line Detection 'In the Field'

Table 6-114: First Line Detection *Leishmania* (Cutaneous).

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Lesion smears of biopsies, scraping, or impression smears	Direct identification of amastigotes in macrophages, Giemsa or HE stained Material from the ulcer base usually has the highest yield	http://www.cdc.gov/dpdx/leishmaniasis/gallery.html	N/A

6.6.12.1.3 Second Line Detection 'In the Lab'

Antibody detection can prove useful in visceral leishmaniasis but is of limited value in cutaneous disease, since most patients do not develop a significant antibody response. In addition, cross reactivity can occur with *Trypanosoma cruzi*, a fact to consider when investigating *Leishmania* antibody response in patients who have been in Central or South America.

Table 6-115: Second Line Detection *Leishmania* (Cutaneous).

Test Method	Sample	Comment	Reference/Company	Code
RT-PCR	Lesion smears of biopsies, scraping, or impression smears	Information on sampling of lesions: http://www.cdc.gov/parasites/leishmaniasis/resources/pdf/cdc_diagnosis_guide_leishmaniasis_2015.pdf	Multiple Multiplex PCR systems have been published, no differentiation within <i>L. donovani</i> -complex (<i>L. donovani</i> and <i>L. infantum</i>). Sequencing necessary in this case.	L

6.6.12.2 Primarily Visceral Manifestation

6.6.12.2.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/leishmania-eng.php>.

<http://www.cdc.gov/parasites/leishmaniasis/>.

DETECTION METHODS

6.6.12.2.2 First Line Detection 'In the Field'

Table 6-116: First Line Detection *Leishmania* (Visceral).

Test Method	Sample	Comment	Reference/Company	Code
Microscopy, but probably not in the field	Buffy coats of peripheral blood, biopsies from lymph nodes or bone marrow	Identification of amastigotes in white blood cells Giemsa stained Low sensitivity in peripheral blood smears, especially in individuals with low parasitemia!! Lymph node biopsy and especially bone marrow is necessary Results are dependent on technical expertise and the quality of prepared slides	http://www.cdc.gov/dpdx/leishmaniasis/gallery.html	N/A
Lateral Flow Assay	Serum (20 µl)	Detects antibodies to members of <i>L. donovani</i> complex	OneStep <i>Leishmania</i> Serum RapiDip™ InstaTest, Diagnostic Automation/Cortez Diagnostics, Inc., US http://www.rapidtest.com/pdf/Leishmania_RapiDiP_176070-1(08-17-2015).pdf	IVD

6.6.12.2.3 Second Line Detection 'In the Lab'

Table 6-117: Second Line Detection *Leishmania* (Visceral).

Test Method	Sample	Comment	Reference/Company	Code
(RT)-PCR	Peripheral blood , buffy coat, spleen or lymph node biopsies, bone marrow aspirates	More sensitive and specific than microscopic examination and parasite culture Remain complex and expensive Subsequent sequencing of amplicates may be necessary for species discrimination (e.g. <i>L. donovani</i> -complex)	18S rRNA followed by conventional hsp70 for sequencing.	L
AB detection: IFA, ELISA, western blot, Direct Agglutination Test (DAT)	EDTA-blood	Depending on the leishmanial species, the ab remain positive for many months, even years, after the patient has been cured and do not differentiate between current and past infection	In house DAT (Tropical Institute, Antwerp) IT-Leish (rK39) , DiaMed, Bio-Rad Laboratories, USA (TI) http://diamedil.info/info/ifo_LEISH.pdf	

Test Method	Sample	Comment	Reference/Company	Code
Antigen detection	Urine		KAtex, Kalon Biological Limited, UK (TI) http://www.kalonbio.co.uk/html/leishmania.html	
Culture		(Cave: this is rather a scientific than a diagnostic tool) Special media is needed Can improve diagnostic sensitivity but is tedious, time-consuming and expensive thus seldom used New culture methods are available		

6.6.13 *Onchocerca Volvulus*

Biosafety Level 2.

6.6.13.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/onchocerca-eng.php>.

<http://www.cdc.gov/parasites/onchocerciasis/index.html>.

6.6.13.2 First Line Detection ‘In the Field’

Table 6-118: First Line Detection *Onchocerca Volvulus*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Skin snips from areas adjacent to nodules (6 snips from different areas of the body)	Identification of microfilariae Skin snips should be thick enough to include the outer part of the dermal papillae but not so thick as to produce bleeding Skin snips should be placed immediately in normal saline or distilled water, just enough to cover the specimen	http://www.cdc.gov/dpdx/onchocerciasis/gallery.html	N/A

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
Microscopy (cont'd)		Microfilariae tend to emerge more rapidly in saline, however in either medium the microfilariae typically emerge in 30 – 60 min and can be seen in wet mount preparations For a definitive diagnosis, allow the wet mount to dry, fix in methanol, and stain with Giemsa or HE		
Lateral Flow Assay	Drop of blood from a fingerstick	Detecting antibodies Test result in 20 minutes For mass screening and surveillance Not intended for diagnosis of active infection, antibodies can remain positive for years	SD Bioline Onchocerciasis IgG4 rapid test, PATH, Standard Diagnostics. http://www.standardia.com/en/home/product/rapid/infectious-disease/Anti-Onchocerciasis_IgG4.html	R

6.6.13.3 Second Line Detection ‘In the Lab’

Microscopy of skin snips remains the golden standard. However, when skin snips are negative and clinical suspicion of infection is high, the general antibody test could be used in an attempt to exclude infection. If the general antibody test were positive, then it might be necessary to consider performing additional skin snips and/or seeking additional diagnostic information by enlisting the assistance of researchers who perform antibody tests.

http://www.cdc.gov/parasites/onchocerciasis/health_professionals/index.html#dx.

Table 6-119: Second Line Detection *Onchocerca Volvulus*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Skin snips	See first line detection method	http://www.cdc.gov/dpdx/onchocerciasis/gallery.html	N/A
Microscopy	Surgically removed nodules	Examined for adult worms	http://www.cdc.gov/dpdx/onchocerciasis/gallery.html#adults	N/A
Slit-lamp examination	Anterior part of the eye	Identify larvae or lesions they cause		N/A
AG or AB detecting immunoassays		There are several <i>Onchocerca</i> -specific serologic tests in existence, but these are currently only		R

Test Method	Sample	Comment	Reference/Company	Code
LIPS (luciferase immunoprecipitation system)		available in the research setting and are not approved for diagnosis in the United States		R

6.6.14 *Paragonimus Westermani*

Biosafety Level 2.

6.6.14.1 PSDS

<http://www.cdc.gov/parasites/paragonimus/>.

6.6.14.2 First Line Detection ‘In the Field’

Detection of eggs in sputum or faeces of patients with paragonimiasis is often very difficult; therefore, serodiagnosis may be very helpful in confirming infections and for monitoring the results of individual chemotherapy.

Table 6-120: First Line Detection *Paragonimus Westermani*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Sputum (unstained)	Identification of eggs in sputum or (more rarely) in feces have a typical morphology with an obvious operculum	http://www.cdc.gov/dpdx/paragonimiasis/gallery.html	N/A
	Feces	Multiple stool exams on different days may be necessary in order to increase sensitivity Cave: Acid-fast stain used for TB testing of sputum may destroy eggs		

6.6.14.3 Second Line Detection ‘In the Lab’

Table 6-121: Second Line Detection *Paragonimus Westermani*.

Test Method	Sample	Comment	Reference/Company	Code
RT-PCR			http://www.biosb.com/biosb-products/real-time-pcr-zoonosis/	

DETECTION METHODS

Test Method	Sample	Comment	Reference/Company	Code
Biopsy	Tissue sample	Search for eggs in a tissue specimen		N/A
	Lung tissue	Adult flukes can be seen	http://www.cdc.gov/dpdx/paragonimiasis/gallery.html#adults	

6.6.15 *Plasmodium Falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, *P. knowlesi*

Biosafety Level 3.

6.6.15.1 PSDS

<http://www.cdc.gov/malaria/index.html>.

6.6.15.2 First Line Detection ‘In the Field’

Malaria has by far the most significant impact as a militarily important disease and deserves special attention. Specific chemoprophylactic regimen is recommended in endemic and hyper endemic areas.

General information on the diagnosis of malaria: <http://www.cdc.gov/dpdx/malaria/dx.html>.

Table 6-122: First Line Detection *Plasmodium* spp.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Minimum 2 ml EDTA blood	Gold standard DiffQuick or Giemsa-stained thick smear or fixed thin blood film – unfixed thick blood film; for final confirmation in a naive individual, repeat testing for three days in a row can be appropriate Cave: the potentially fatal species <i>P. falciparum</i> and <i>P. knowlesi</i> cannot be differentiated by microscopy (identical morphology of trophozoites)	http://www.cdc.gov/dpdx/malaria/gallery.html	N/A
Lateral Flow Assay	Drop of blood	Antigen (HRP2 or LDH) detecting immunochromatographic test strips. Negative results must be confirmed by thin / thick smear microscopy Rapid test SD Malaria Ag Pf/Pan POCT very accurate for <i>P. falciparum</i> , less accurate for other species	SD Malaria Ag Pf/Pan POCT http://www.standardia.com/en/home/product/rapid/infectious-disease/Malaria_Ag_PfPan.html BinaxNOW® http://www.alere.com/us/en/product-details/binaxnow-malaria.html	D

Test Method	Sample	Comment	Reference/Company	Code
Lateral Flow Assay (cont'd)		Important remark: Inactivation techniques (for example with suspected ebola samples) may interfere with the BinaxNOW® test The inactivation technique has to be validated with the test method		

6.6.15.3 Second Line Detection ‘In the Lab’

Microscopy (morphologic analysis) continues to be the “gold standard” for malaria diagnosis.

Table 6-123: Second Line Detection *Plasmodium* spp.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Minimum 2 ml EDTA blood	See first line detection method		N/A
RT-PCR	EDTA-blood	To differentiate between species in cases where morphologic characteristics overlap (especially <i>Plasmodium vivax</i> and <i>P. ovale</i>), as well as in cases where parasite morphology has been altered by drug treatment or improper storage of the sample In addition, molecular tests such as PCR can detect parasites in specimens where the parasitemia may be below the detectable level of blood film examination	Malaria Parasites Real-time PCR kit (Liferiver Bio SB) or separate kits for the different species (Liferiver Bio SB) Cnops <i>et al.</i> [14]	L

6.6.16 *Schistosoma Mansoni*, *S. haematobium*, *S. japonicum*

Biosafety Level 2.

6.6.16.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds136e-eng.php>.

<http://www.cdc.gov/parasites/schistosomiasis/index.html>.

DETECTION METHODS

6.6.16.2 First Line Detection ‘In the Field’

Table 6-124: First Line Detection *Schistosoma* spp.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stools (<i>S. mansoni</i> + <i>japonicum</i>), Urine (<i>S. haematobium</i>)	<i>S. haematobium</i> + <i>japonicum</i> : eggs pass intermittently and in small amounts only after 25 to 60 days post-infection, test can be done on stool as such or after concentration by sedimentation <i>S. haematobium</i> : Concentration of urine by sedimentation or filtration, daily peak of eggs in urine between 10 AM and 02 PM, best found in last part of micturition, not always present, repeating of exam is important, present in urine 6 to 8 weeks post infection	http://www.cdc.gov/dpdx/schistosomiasis/gallery.html#mansoni http://www.cdc.gov/dpdx/schistosomiasis/gallery.html#japoneggs http://www.cdc.gov/dpdx/schistosomiasis/gallery.html#haemaeggs	N/A

6.6.16.3 Second Line Detection ‘In the Lab’

Because test sensitivity with ELISA assays is reduced for species other than *S. mansoni*, immunoblots of the species appropriate to the patient’s travel history are also tested to ensure detection of *S. haematobium* and *S. japonicum* infections.

Table 6-125: Second Line Detection *Schistosoma* spp.

Test Method	Sample	Comment	Reference/Company	Code
ELISA	10 µl serum	Minimum 8 weeks post exposure (earlier than stools and urine); serology recommended in case of negative microscopy but clinical suspicion and after exposure	Anti- <i>Schistosoma mansoni</i> IgG Human ELISA Kit http://www.abcam.com/ps/products/108/ab108769/document/s/ab108769%20Anti-Schistosoma%20mansoni%20IgG%20Human%20ELISA%20Kit%20(web%20site).pdf Human <i>Schistosoma</i> <i>haematobium</i> antibody (IgG) ELISA Kit http://www.mylabsource.com/images/tds/protocol_others/MB930732.pdf	R

Test Method	Sample	Comment	Reference/Company	Code
ELISA (cont'd)			<i>Schistosoma haematobium</i> Antibody (IgG) ELISA Kit http://www.antibodies-online.com/kit/858249/Schistosoma+Haematobium+IgG+ELISA/	
Biopsy	Rectum (superficial)	Detection of eggs	http://www.cdc.gov/dpdx/schistosomiasis/gallery.html#mansoni http://www.cdc.gov/dpdx/schistosomiasis/gallery.html#japon eggs	N/A
ELISA	5 µl serum or plasma	For <i>Schistosoma spp.</i>	AccuDiag™ <i>Schistosoma</i> IgG ELISA Kit http://www.rapidtest.com/pdf/Schistosoma_8209-35(12-12-2013).pdf	R

6.6.17 *Taenia Saginata*

Biosafety Level 2.

6.6.17.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/taenia-saginata-eng.php>.

<http://www.cdc.gov/parasites/taeniasis/index.html>.

6.6.17.2 First Line Detection ‘In the Field’

Table 6-126: First Line Detection *Taenia Saginata*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stools or anal ‘scotch test’	Gravid proglottids can be seen (macroscopically) with or without defecation; eggs can be detected in stools or on the scotch test, from 10 to 12 weeks post infestation Microscopic identification of gravid proglottids (or, more rarely, examination of the scolex) allows species determination	http://www.cdc.gov/dpdx/taeniasis/gallery.html http://www.cdc.gov/dpdx/taeniasis/dx.html	N/A

DETECTION METHODS

6.6.17.3 Second Line Detection ‘In the Lab’

Table 6-127: Second Line Detection *Taenia Saginata*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stools or anal ‘scotch test’	See first line detection		N/A
ELISA		Antibody detection may prove useful especially in the early invasive stages, when the eggs and proglottids are not yet apparent in the stools		

6.6.18 *Taenia Solium* (Taeniasis)

Biosafety Level 3.

6.6.18.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/taenia-solium-eng.php>.

<http://www.cdc.gov/parasites/taeniasis/index.html>.

6.6.18.2 First Line Detection ‘In the Field’

Table 6-128: First Line Detection *Taenia Solium* (Taeniasis).

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stools	Gravid proglottids and eggs can be detected in stools 5 to 12 weeks post infestation.	http://www.cdc.gov/dpdx/taeniasis/gallery.html http://www.cdc.gov/dpdx/taeniasis/dx.html	N/A

6.6.18.3 Second Line Detection ‘In the Lab’

Table 6-129: Second Line Detection *Taenia Solium* (Taeniasis).

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Stools	See first line detection		N/A
ELISA	Plasma or serum	Antibody detection may prove useful especially in the early invasive stages, when the eggs and proglottids are not yet apparent in the stools	http://www.abcam.com/Taenia-solium-IgG-Human-ELISA-Kit-ab108770.html	R

6.6.19 *Taenia Solium* (Cysticercosis)

Biosafety Level 3.

6.6.19.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/taenia-solium-eng.php>.

<http://www.cdc.gov/parasites/taeniasis/index.html>.

6.6.19.2 First Line Detection ‘In the Field’

No first line detection methods exist.

6.6.19.3 Second Line Detection ‘In the Lab’

Diagnosis is most often made by MRI or CT scans; addition of serologic screening by ELISA with confirmation by Western blot increases sensitivity of diagnosis of cysticercosis.

http://www.cdc.gov/parasites/cysticercosis/health_professionals/index.html.

Table 6-130: Second Line Detection *Taenia Solium* (Taeniasis).

Test Method	Sample	Comment	Reference/Company	Code
MRI, CT scan	N/A	Patients that are symptomatic will undergo imaging first, serology will be used to diagnose cysticercosis		N/A
ELISA	Serum, CSF		GenWay <i>T. solium</i> IgG ELISA http://www.genwaybio.com/elisas/infectious-disease-elisa/parasites-elisa/taenia-solium-igg-elisa	R
	Serum		Cysticercosis Serum Microwell Elisa, IVD Research, USA Cysticercosis Ag ELISA, Apdia, Belgium	
	CSF	Especially for neurocysticercosis	http://www.apdiagroup.com/images/pdf/elisaassays/Cysticercosis/14-8951%20apDia%20Leaflet%20cysticercose_LR_v03.pdf	D
Western blot	Serum, CSF	Confirmation of ELISA		

DETECTION METHODS

6.6.20 *Toxoplasma Gondii*

Biosafety Level 2.

6.6.20.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds153e-eng.php>.

<http://www.cdc.gov/parasites/toxoplasmosis/index.html>.

6.6.20.2 First Line Detection ‘In the Field’

No first line detection methods exist.

6.6.20.3 Second Line Detection ‘In the Lab’

<http://www.cdc.gov/dpdx/toxoplasmosis/dx.html>.

Table 6-131: Second Line Detection *Toxoplasma Gondii*.

Test Method	Sample	Comment	Reference/Company	Code
Serology		IgG and IgM	Many manufacturers provide serological test kits for <i>Toxoplasma</i> (for example Siemens Enzygnost)	
Commercial PCR	Blood, CSF, stools		http://www.biosb.com/wp-content/uploads/243-Toxoplasma-Gondii-Real-Time-PCR-Kit-ZD-0075-02.pdf	IVD
Medical imaging		Fond of the eye: For chorioretinitis in case of ocular toxoplasmosis	http://www.cdc.gov/dpdx/toxoplasmosis/gallery.html	N/A
Histology		Tachyzoites (trophozoites) of <i>Toxoplasma gondii</i> are approximately 4 – 8 µm long by 2 – 3 µm wide, with a tapered anterior end, a blunt posterior end and a large nucleus They may be found in various sites throughout the body of the host	http://www.cdc.gov/dpdx/toxoplasmosis/gallery.html	N/A

6.6.21 *Trichinella Spiralis*, *Trichinella spp.*

Biosafety Level 2.

6.6.21.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/trichinella-eng.php>.

<http://www.cdc.gov/parasites/trichinellosis/>.

6.6.21.2 First Line Detection ‘In the Field’

Suspicion of infection is based on history, clinical symptoms and eosinophilia.

Acute phase (after ingestion of contaminated, infectious meat) is potentially fatal. This is prior to antibody production. In this case microscopy of *T. spiralis* larvae in blood samples is recommended.

6.6.21.3 Second Line Detection ‘In the Lab’

The suspicion of trichinellosis (trichinosis), based on history, clinical symptoms, and eosinophilia, can be confirmed by specific diagnostic tests, including antibody detection, muscle biopsy, and microscopy.

<http://www.cdc.gov/dpdx/trichinellosis/dx.html>.

Table 6-132: Second Line Detection *Trichinella Spiralis*, *Trichinella spp.*

Test Method	Sample	Comment	Reference/Company	Code
IgG ELISA	Serum	Cross-reactions with other nematodes are possible Antibody levels are often not detectable until 3 to 5 weeks post-infection, well after the onset of acute-stage illness Multiple serum specimens should be drawn several weeks apart to demonstrate seroconversion in patients whose initial specimen was negative IgG, IgM, and IgE antibodies are detectable in many patients; however, tests based on IgG antibodies are most sensitive	AccuDiag™ <i>Trichinella</i> IgG ELISA Kit http://www.rapidtest.com/pdf/Trichinella%20IgG%208207-35%20(10-18-2013).pdf <i>Trichinella</i> Antibody Detection Test, <i>Trichinella</i> Microwell Serum ELISA, SCIMEDX Corporation, USA http://www.scimedx.com/pr/SciMedx_Acquires_IVD.pdf	D
Biopsy	Muscle		http://www.cdc.gov/dpdx/trichinellosis/gallery.html	N/A

6.6.22 *Trypanosoma Cruzi*

Biosafety Level 3.

6.6.22.1 PSDS

<http://www.cdc.gov/parasites/chagas/>.

DETECTION METHODS

6.6.22.2 First Line Detection ‘In the Field’

Table 6-133: First Line Detection *Trypanosoma Cruzi*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Blood (wet smears)	Only during the acute stage of the disease detection of trypomastigotes is easy to perform but much less numerous when compared with <i>T. brucei ssp.</i>	http://www.cdc.gov/dpdx/trypanosomiasisAmerican/gallery.html	N/A

6.6.22.3 Second Line Detection ‘In the Lab’

Table 6-134: Second Line Detection *Trypanosoma Cruzi*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Blood (wet smears), CSF	Only in the acute stage of the disease	http://www.cdc.gov/dpdx/trypanosomiasisAmerican/gallery.html	N/A
Serology	Serum or plasma	IFA and EIA; two serological tests of different methodology needed for diagnosis	Bioelisa Chagas, biokit, Spain http://www.biokit.com/productos/reagents/bioelisa/others/bioelisa-chagas.aspx	D
Biopsy			http://www.cdc.gov/dpdx/trypanosomiasisAmerican/gallery.html	

6.6.23 *Trypanosoma Brucei Gambiense* and *Rhodesiense*

Biosafety Level 2.

6.6.23.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/msds158e-eng.php>.

<http://www.cdc.gov/parasites/sleepingsickness/biology.html>.

6.6.23.2 First Line Detection ‘In the Field’

Table 6-135: First Line Detection *Trypanosoma Brucei Gambiense* and *Rhodesiense*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Blood smear	Especially numerous trypomastigotes during the acute stage of the disease; detection of trypomastigotes is easy to perform even without Giemsa staining due to their motility	http://www.cdc.gov/dpdx/trypanosomiasisAfrican/gallery.html	N/A

6.6.23.3 Second Line Detection ‘In the Lab’

Table 6-136: Second Line Detection *Trypanosoma Brucei Gambiense* and *Rhodesiense*.

Test Method	Sample	Comment	Reference/Company	Code
IF	Serum	For IgG	In house IFAT (Institute for Tropical Medicine, Antwerp, Belgium)	
CATT	Serum	For total Ig	In house prepared (Institute for Tropical Medicine, Antwerp, Belgium)	

6.6.24 *Wuchereria Bancrofti*, *Brugia Malayi*, *B. timori*, *Loa Loa*

Biosafety Level 2.

6.6.24.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/wuchereria-bancrofti-eng.php>.

<http://www.cdc.gov/parasites/lymphaticfilariasis/>.

6.6.24.2 First Line Detection ‘In the Field’

http://www.cdc.gov/dpdx/resources/pdf/benchAids/Wbancrofti_Lloa_benchaid_who.pdf.

Table 6-137: First Line Detection *Wuchereria Bancrofti*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Blood smears (thick or thin stained with Giemsa or HE)	Nocturnal activity of the parasites, except <i>Loa Loa</i> (day-active): if nocturnal, blood best collected at night (10 PM – 2 AM) For increased sensitivity, concentration techniques can be used	http://www.cdc.gov/dpdx/lymphaticFilariasis/gallery.html http://www.cdc.gov/dpdx/lymphaticFilariasis/dx.html	N/A
Immuno-chromatographic Card Test (ICT)	Drop of whole blood, serum or plasma		BinaxNOW® ICT Filariasis 25T Kit http://www.alere.com/ww/en/product-details/binaxnow-filariasis.html	IVD

DETECTION METHODS

6.6.24.3 Second Line Detection ‘In the Lab’

Table 6-138: Second Line Detection *Wuchereria Bancrofti*.

Test Method	Sample	Comment	Reference/Company	Code
Microscopy		See first line detection		N/A
ELISA		For FILARIOSE, not specific <i>Wuchereria</i>	<i>Acanthocheilonema viteae</i> , Bordier Affinity Products SA, Switzerland http://www.bordier.ch/9400%20Acanthocheilonema%20viteae/index.htm	D

6.6.25 *Toxocara Canis, T. cati*

Biosafety Level 2.

6.6.25.1 PSDS

<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/toxocara-eng.php>.

<http://www.cdc.gov/parasites/toxocariasis/>.

6.6.25.2 First Line Detection ‘In the Field’

In this parasitic disease the diagnosis does not rest on identification of the parasite. Since the visceral larva migrans (L2 larval stage) does not develop into adults in humans, a stool examination would not detect any *Toxocara* eggs.

No first line detection methods available.

6.6.25.3 Second Line Detection ‘In the Lab’

Table 6-139: Second Line Detection *Toxocara Canis, T. cati*.

Test Method	Sample	Comment	Reference/Company	Code
ELISA	Serum (minimum 0,5 ml) or vitreous humour (for ocular toxocariasis)	IgG antibody test against larval excretory/secretory antigen Cross-reactions with strongyloidiasis, trichinosis, amoebiasis and fascioliasis Positive tests are confirmed with Western Blot	Abcam (anti- <i>Toxocara canis</i>) http://www.abcam.com/ps/products/108/ab108775/document/s/ab108775%20Anti-Toxocara%20canis%20IgG%20Human%20ELISA%20Kit%20(web%20site).pdf <i>Toxocara canis</i> , Bordier Affinity Products SA, Switzerland http://www.bordier.ch/9200%20Toxocara%20canis/index.htm	R D

6.6.26 *Strongyloides Stercoralis*, *Strongyloides spp.*

Biosafety Level 2.

6.6.26.1 PSDS

<http://www.cdc.gov/parasites/strongyloides/index.html>.

6.6.26.2 First Line Detection ‘In the Field’

Table 6-140: First Line Detection *Strongyloides Stercoralis*, *Strongyloides spp.*

Test Method	Sample	Comment	Reference/Company	Code
Microscopy	Fresh stools (max. 5 hrs), minimum 20 gr	Detection of L1 larvae, less frequent eggs and L2 larvae in feces, after concentration (for example Baermann method) Repeated tests may be necessary due to intermittent expulsion of <i>Strongyloides</i> Might be challenging for inexperienced lab workers under field conditions due to need for concentration methods and repeated testing	http://www.cdc.gov/dpdx/strongyloidiasis/gallery.html http://www.cdc.gov/dpdx/strongyloidiasis/dx.html	N/A

6.6.26.3 Second Line Detection ‘In the Lab’

Table 6-141: Second Line Detection *Strongyloides Stercoralis*, *Strongyloides spp.*

Test Method	Sample	Comment	Reference/Company	Code
Microscopy		See first line detection		
RT-PCR	Stool		Verweij <i>et al.</i> [98]	L
ELISA	Serum	IgG Indicated when the infection is suspected and the organism cannot be demonstrated by duodenal aspiration, string tests, or by repeated examinations of stool Cross-reactions in patients with filariasis and some other nematode infections may occur Antibody test results cannot be used to differentiate between past and current infection	AccuDiag™ <i>Strongyloides</i> IgG ELISA Kit http://www.rapidtest.com/index.php?i=Strongyloides-ELISA-kit&id=168&cat=17 <i>Strongyloides ratti</i>, Bordier Affinity Products SA, Switzerland http://www.bordier.ch/9450%20Strongyloides%20ratti/index.htm	IVD



Chapter 7 – SURVEILLANCE SYSTEM

The high importance of infectious diseases for military missions abroad requires the implementation of lab-based diagnostic results into NATO disease surveillance systems to immediately provide relevant information to the chain of command. The establishment of a “near real-time database surveillance” with direct uploading of test results into operational NATO surveillance systems supports FHP by enabling epidemiological risk assessments and implementation of preventive measures.

To date, no communication platform exist between NATO labs. Currently the exchange of knowledge and methods depends mostly on personal communication. The connection between and to existing surveillance systems should be established to share knowledge and data. The RTG HFM-230 working group focused on the detection of infectious pathogens in men. This is of high importance for the detection of specific diseases in ill persons. This information should be integrated in existing surveillance systems, e.g.:

- EPI-NATO surveillance system = syndromic disease surveillance (limited data, not based on the detection of a pathogen).
- **Deployment health surveillance (AMedP 21) for Real-time syndromic Disease surveillance.**
- FRA ASTER (Real-time syndromic surveillance) as an example on humans (example of an established system that works, but only on a national level).

The NATO surveillance system must be able to connect data and assessments from different resources and data bases.

In addition internal NATO surveillance systems should use or refer to civilian surveillance and reporting systems like WHO, CDC, ECDC, ProMedMail, OIE, FAO, ENIVD or SurSaUD (Surveillance Sanitaire des urgences et des décès). These surveillance/reporting systems have usually the drawback, that they are usually voluntary and susceptible to political or economic interests or technical as well as systemic problems. The connection to NATO-internal intelligence capabilities prior to the mission will definitely support the more specified planning and resource management for diagnostic capabilities. Currently all NATO surveillance systems are syndrome-based, laboratory confirmed diseases are not mentioned. These capabilities inform of potential therapeutic needs on site but they may be too late to implement preventive measures. During infectious disease outbreaks, diagnostic testing clearly supports MTF’s decision on therapy and containment during outbreak management.

The implementation of surveillance systems prior to or immediately after the beginning of the mission will provide a scientific basis for specific preventive measures. Current surveillance systems lack data concerning possible reservoirs and vectors. An additional surveillance system should be established for testing of reservoirs and vectors to provide improved site risk estimates, protect military member’s health and provide focused FHP interventions.



Chapter 8 – GAP ANALYSIS

8.1 LACK OF A NETWORK AND COMMUNICATION PLATFORM BETWEEN NATO LABORATORIES

Within NATO, no database exists with details on all existing NATO laboratories and their capabilities. That information would be very useful for medical or veterinary personnel and perhaps life-saving in the field of operation in case of problematic detection of pathogen or in an outbreak situation.

Moreover, a real network of NATO laboratories does not exist at this time to exchange scientific and diagnostic knowledge, enable cooperation or compare the quality and reliability of diagnostic tests. There is no official communication channel or system through which these NATO laboratories could communicate. Currently, all cooperation is performed on personal level or bi-party laboratory cooperation.

8.2 LACK OF CONNECTION BETWEEN EXISTING SURVEILLANCE SYSTEMS

To date, no laboratory-based surveillance system exists on a NATO-level to enable the implementation of specific preventive and Force Health Protection measures. The existing surveillance system, EPINATO, is syndrome-based and does not provide information on clinical diagnosis, nor on the pathogens involved.

At the same time, no overall NATO outbreak alert system exists that could be used by the majority of NATO countries and that could provide a formal communication channel and discussion platform in case of an outbreak emergency.

8.3 NEWLY (RE-)EMERGING PATHOGENS REQUIRES A REGULAR REVISION

The data for the priority list were collected until 2014 and the data for the detection methods until 2015. Therefore not all new or newly re-emerging pathogens are fully discussed in this report. For example, MERS detection methods are listed however it was not scored for the priority list. Zika virus re-emerged too recently to be included in the list, but deserves attention in a possible follow-up to this work.

8.4 PRIORITIZATION REQUIRES A REGULAR REVISION

The priority list does not provide a definite answer on pathogen prioritization as it is only a summary of individual lists that were prepared by experts or expert groups in each participating country. As such it should not be interpreted as an absolute fact but rather as a starting point for discussion. It may also be used together with epidemiology reports in area of operations.

Only eight NATO countries participated in RTG HFM-230, so there is a chance that the priority list and detection methods do not reflect the situation for the whole NATO alliance.

Advances in knowledge about pathogens, and development of new therapeutics or prophylaxis might have an influence on the scoring in the future.

8.5 DEVELOPMENT OF NEW DETECTION METHODS REQUIRE A REGULAR REVISION

The list of detection methods does not cover all methods that exist for an individual pathogen. It is only a recommendation from RTG HFM-230 participating countries how to approach detection of listed pathogens in the field laboratory and in the second line laboratory.

Method classification is not definite and it will change in time.

At this moment, a fast and reliable method that can be performed by people with little or no experience does not exist for each pathogen. Development of newer and easier methods is ongoing, so a regular revision of methods is necessary.

8.6 NON-ZOONOTIC FOOD AND WATERBORNE PATHOGENS ARE NOT MENTIONED (ARTIFICIAL DISTINCTION)

Most laboratories or military units that deal with zoonotic and vector-borne pathogens are also responsible for non-zoonotic food- and waterborne pathogens. This distinction was made at the start of the Working Group, however this might be artificial and might need revision in a further project.

Chapter 9 – RECOMMENDATIONS

9.1 INSTALLATION OF A CONTINUOUS EXPERT PANEL

As it was predicted by Charles Nicolle's in 1930 [40], in the natural history of diseases, some are disappearing and other emerge continuously. In spite of the progress of science and technology, it still remains impossible to say when the next epidemic will start and which pathogen will be responsible for it. Even for recognized experts, the recent outbreaks of Zika, Ebola or MERS constituted a surprise. Given the frequency of new and re-emerging infectious diseases, it is necessary to regularly review the priority list. Additionally, the regular development of new testing methodologies requires regular updates to the detection methods.

Further for some pathogens (antigen) detection in humans might be insufficient during the infection as the onset of symptoms develops after the viremic, bacteremic or parasitic phase. In these cases serological investigations might confirm the microbe but the antigen itself cannot be further investigated. This might be of high importance in new outbreak scenarios, uncommon outbreaks, etc. Therefore in addition to the detection of the pathogen in humans, investigation of possible vectors such as ticks, fleas or mosquitoes or reservoirs (e.g., rodents, birds) is needed in order to obtain an in-depth overview on the responsible pathogens. The RTG HFM-230 Task Group therefore recommends to establish an expert panel that is able to assess and investigate natural reservoirs of zoonotic and vector-borne pathogens in military context.

9.2 ADDITIONAL NATO COUNTRIES OR NON-NATO COUNTRIES SHOULD BE INVOLVED IN THIS WORK

In the field of detection methods of pathogens, sharing information between countries is critical. Developed in-house test may differ from other labs and may be useful for other NATO countries. The participation of other countries should be considered for the follow-up of this work, in order to have a broader and more relevant source of data.

9.3 CREATE A NATO-WIDE LABORATORY NETWORK

During this four-year project, relationships were fostered or reinforced between the labs of the participating countries. Each meeting provided the opportunity to visit one or more laboratories within the host country, to include civilian as well as military. This network is now very efficient to exchange information and even provide support for analysis, in case of an unusual situation. These relationships should be maintained, reinforced and enlarged in the future, for the benefit of all.

To enhance the collaboration inside NATO, a laboratory network could be installed. At a minimum, a list of military labs with a description of their main objectives, areas of interest and contact details would be useful.

9.4 SURVEILLANCE SYSTEM IN ANIMAL POPULATIONS

Different surveillance systems are operated to monitor the health of military and civilian populations but to our knowledge, no animal surveillance system exists at the national or international level, for the armed forces. However we know that under appropriate conditions, monitoring domestic and wild animal populations can be helpful to provide early warning of health risks for troops. Animals and humans share the same environment

RECOMMENDATIONS

where the same pathogens are circulating, sometimes via arthropod vectors. Some pathogens can persist for a long time in the environment (in the soil, water, or air). The use of animals as sentinels is under-utilized although it appears particularly informative during the initial stages of deployment in foreign countries, as local animals have already been exposed to the concerned pathogens. Sentinel animals, either domestic or wild, are useful when clinical signs in these populations can be detected before human illness emerged, or soon enough to allow preventive measures to be initiated. Active surveillance for sporadic illness in animals could help in detecting ongoing exposure risks. Surveillance of sentinel animals completes and strengthens epidemiological surveillance conducted on the human populations and contributes to medical intelligence.

9.5 DEVELOP A WEBPAGE INSIDE THE NATO WEBSITE TO CREATE A COMMUNITY AND SHARE THE INFORMATION

In order to maintain the links between the member countries, to share updated versions of the documents produced and to provide an access anytime anywhere, development of a RTG HFM-230 webpage would allow member countries to maintain links to one another, and provide easy access to share updated versions of the documents produced.

9.6 DEVELOP A NATO-BASED OUTBREAK ALERT SYSTEM

The relevance and feasibility of a NATO-based outbreak alert system should be further studied in order to check if this would contribute to existing networks and systems.

9.7 DEVELOP A ‘ONE HEALTH’ PANEL WITHIN NATO

‘One Health’ recognizes that the health of humans, animals and ecosystems are interconnected. It involves applying a coordinated, collaborative, multidisciplinary and cross-sectoral approach to address potential or existing risks that originate at the animal-human-ecosystems interface. Within NATO, such an approach could become more concrete with the creation of a devoted working group, implementing a multidisciplinary dynamic in the different fields of ‘One Health’.

9.8 FURTHER STRENGTHEN CIVILIAN-MILITARY COLLABORATION ON OUTBREAK SURVEILLANCE AND DIAGNOSTICS OF VECTOR-BORNE AND ZOONOTIC DISEASES

In 2006, an agreement was signed between the world organization for animal health (OIE) and the International committee of military Medicine (ICMM). The two organizations agreed on a reciprocal representation, the exchange of information and documents and a cooperation on questions of mutual interest. Such an agreement could be reached between OIE and NATO, for the benefit of the two organizations.

9.9 COMMUNICATE RELEVANT INFORMATION TO THE LOCAL AUTHORITIES IN CHARGE OF HUMAN AND ANIMAL HEALTH

When a pathogen of importance for human and / or animal health is identified in a country of deployment, this information should systematically be communicated to the local authority. Indeed there are no barriers for

pathogens between military and civilians populations and the management of an outbreak requires a global approach. In addition the presence of some pathogens (list of reportable diseases) must be reported to the World Health Organization for the World Organization for Animal Health and it is a responsibility of each individual country.

9.10 MICROSCOPY SHOULD BE INCLUDED IF POSSIBLE IN THE LOWEST LEVEL FIELD LAB (WITH EXPERIENCED PERSONNEL)

The microscopic examination is essential in the detection of pathogens, most particularly parasites. A microscope should be included for the more basic field laboratory and staff should be carefully trained on microscopy.

RECOMMENDATIONS



Chapter 10 – REFERENCES

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Annex A – TECHNICAL ACTIVITY PROPOSAL (TAP)

ACTIVITY REFERENCE NUMBER	HFM	ACTIVITY TITLE Development of depository of fast and reliable detection methods for zoonotic agents.	APPROVAL 2012
TYPE AND SERIAL NUMBER	RTG-230		START 2012
LOCATION(S) AND DATES		1 st Mtg: Neuilly-sur-Seine, FRA, 29 May – 1 Jun 2012 2 nd Mtg: Rome, ITA, 17 – 20 Dec 2012 3 rd Mtg: Ljubljana, SVN, 30 Sep – 5 Oct 2013 4 th Mtg: Rome, ITA, 24 – 28 Mar 2014 5 th Mtg: Prague, CZE, 24 – 28 Nov 2014 6 th Mtg: Ghent, BEL, 4 – 8 May 2015 7 th Mtg: Ljubljana, SVN, 7 – 11 Sep 2015 8 th Mtg: Munich, DEU, 23 – 26 Feb 2016	END 2016
COORDINATION WITH OTHER BODIES		HFSC, COMED, BioMEDAC, VETMED	
NATO CLASSIFICATION OF ACTIVITY		UU	Non-NATO Invited Yes
PUBLICATION DATA		TR	
KEYWORDS	Zoonoses, bio-terrorism, serology, molecular biology, virus, bacteria.		

I. BACKGROUND AND JUSTIFICATION (RELEVANCE TO NATO)

A zoonosis is any infectious disease that can be transmitted from non-human animals, both wild and domestic, to humans. Zoonoses have been known since early historical times (i.e. plague, anthrax). Of the 1415 pathogens known to affect humans, 61% are zoonotic. The interdisciplinary field of human and veterinary medicine is already largely concerned with zoonoses as outbreaks of (re-)emerging pathogens regularly occur (e.g., Ebola, Lassa, Nipah, Hendra viruses, new Influenza strains, EHEC strains, some Salmonella strains). Zoonoses are of military interest because they are often emerging or even unrecognized diseases, or have increased virulence in populations lacking immunity. Moreover, one of the major factors contributing to the appearance of new zoonotic pathogens in human populations is increased contact between humans and wildlife. Deployed troops are thus at risk for zoonotic diseases, while the risk of pathogen transfer from a deployment area to the nation of origin is also a concern. Infectious diseases acquired in the operation zone do still account for more military hospital admissions than battlefield injuries. Whilst these arguments are valid for natural acquired pathogens, consideration should be given to the use of zoonotic agents as a possible biological weapon, the list of agents that can be a candidate biological weapon consists for 70% of zoonotic pathogens. The easy access to these agents in nature is a serious problem for biological weapons proliferation and is increasing the probability of a serious bio-terrorism incident. Concerted action for detection and surveillance is thus of importance, together with dissemination of data and information between NATO partners.

II. OBJECTIVE(S)

The RTG will define standards for first line (“in the field”) and second line (“in the lab”) detection and identification methods for viral and bacterial zoonoses. The RTG will also review the outbreak alert system and on-sight surveillance and create a list of pathogen collections of which items can serve as positive control or for validation purposes.

III. TOPICS TO BE COVERED

- Depository of fast, reliable detection and identification methods for viral zoonoses.
- Depository of fast, reliable detection and identification methods for bacterial zoonoses.
- Charting existing pathogen collections / defining priority pathogens.
- Outbreak alert communication.
- On-sight surveillance of priority pathogens.

IV. DELIVERABLE (E.G., S/W ENGAGE MODEL, DATABASE, ETC.) AND/OR END PRODUCT (E.G., FINAL REPORT)

- Annual report.
- Technical reports (final RTG report).
- Reviews of the literature will be published in peer-reviewed (military) scientific journals.
- NATO symposium.
- International symposium.

V. TECHNICAL TEAM LEADER AND LEAD NATION

Technical Team Leader: Med Lt Col C. Vandenvelde (BEL) and Mr. P. Heyman (BEL).

Lead Nation: BEL.

VI. NATIONS WILLING/INVITED TO PARTICIPATE

Nations that participated in the ET-118: BEL, DEU, FRA, USA, TUR, ROU, SVN.

Other NATO nations, non-NATO nations, PfP nations, Mediterranean Dialogue nations and Contact Nations which are willing to participate are kindly invited to contact the Technical Team Leaders.

VII. NATIONAL AND/OR NATO RESOURCES NEEDED (PHYSICAL AND NON-PHYSICAL ASSETS)

Individual nation's resources will be required to support RTG members' travel and participation in RTG meetings.

VIII. CSO RESOURCES NEEDED (E.G., CONSULTANT FUNDING)

CSO support for symposium. CSO support for consultants (2 – 3).



Annex B – TERMS OF REFERENCE (TOR)

I. ORIGIN

A. Background

Zoonotic diseases are defined as diseases transmitted -directly or indirectly- from vertebrate animals to humans, while vector-borne diseases are diseases primarily transmitted by arthropod bites. Many of these diseases are significant for military operations and are closely related to instability, conflict, war and environmental disasters. Refugees and displaced persons may further spread zoonotic and vector-borne disease agents into formerly non-endemic areas. Deploying troops into those areas will significantly increase their exposure and infection rate and directly results in decreased combat effectiveness and troop readiness.

Additionally, several factors may be responsible for the acceleration of the emergence of new zoonotic pathogens during the past decades. The ever growing human and livestock animal populations that in turn induce increasingly close contact of people and animals, increased travel and transportation speed making it today possible to go around the globe in less than the incubation period of most infectious agents as well as human-induced ecological, eco-niche and environmental changes. Bioterrorist activities (supported by rogue governments or originating from educated amateurs) become increasingly likely. A multitude of parameters might contribute to the emergence of a new zoonotic disease: mutation, natural selection, and evolutionary progression of viruses and bacteria; transmission and adaption to new hosts; acquired immunity by infection or vaccination, behavioral features as well as ecologic and climatologic factors.

During the past two decades a number of new zoonotic and vector-borne diseases have emerged, while many old ones have resurged. Many of these agents also deserve attention because they are potential bioweapons. The above mentioned factors contributed to the rapid spread of disease into new areas and infections by several of these agents may result in high mortality. For example, West Nile Virus (WNV) not only expanded its distribution range in Europe but in 1999 was also introduced and rapidly spread throughout the Americas.

An example of indirect transmission of zoonotic agents would be the food- and water-borne infections due to the ingestion of pathogenic bacteria, such as entero-haemorrhagic *Escherichia coli* or *Salmonella* has dramatically increased. Increasing populations of production animals and wildlife, together with modern breeding methods for domestic animals favored the spread of pathogens. The increased number of pets in developed countries and the importation of exotic wildlife species have resulted in the emergence of new zoonotic agents. Population displacement (human or animal, voluntary or not) or translocation via game release, and international agricultural trade, have led to the emergence of zoonoses. Increased human contacts with wildlife reservoirs, associated with military operations as well as with leisure activities (hunting, fishing, tourism) may expose humans to zoonotic agents excreted by animal carriers or to arthropod vectors.

Zoonotic parasitic diseases are transmitted to humans by ingesting spores, cysts, oocysts, ova, larval and encysted stages, mainly by eating raw or undercooked meat containing infective tissue stages. A number of parasites can also be transmitted by arthropod vectors. Humans may be final, intermediate or accidental hosts. The water-food connection for parasitic zoonoses has faeces as a major vehicle for many environmental transmissible stages (e.g., *Echinococcus spp.*), only few pathogens contaminate the environment via urine. The contamination by the various stages can be direct or indirect. The disposal of animal (and human) faeces remains a significant operational problem in many countries.

B. Justification (Relevance for NATO)

Bacterial, parasitic and viral zoonoses have had their influence on military campaigns throughout history, at times playing an important role in determining the result. Bacterial and viral agents for possible use in biological warfare include the organisms causing anthrax, plague, tularemia, brucellosis, glanders, melioidosis, various food borne illnesses, cryptosporidiosis, cryptococcosis, Q fever, psittacosis, dengue fever, smallpox, viral equine encephalitides, and the viral hemorrhagic fever group. Except for smallpox and dengue fever, all are commonly found in animals. A report in the Journal of the American Medical Association /Nature concluded that 80% of the pathogens likely to be used in bio-warfare are zoonotic. As a rule, only basic microbiological skills and equipment are needed to produce bioterrorist agents. Several zoonotic pathogens are among the most likely biological weapon threats, particularly because of their stability in aerosols, their virulence and infectivity if acquired through inhalation (e.g. *Y. pestis*, *C. burnetii*, *F. tularensis*). Aerosols can also be self-inflicted, for example propellers and engine motors can bring massive quantities of infected dust in the air in specific endemic regions. The potency, the cost-effectiveness, and ease of use guarantees that zoonotic agents remain a constant threat to public health in the form of bio-weapons. Another detrimental effect of the use of zoonotic agents is that they can persist in the environment (in the soil, water, or air). Sentinel animals can serve as an early warning to humans if clinical signs are detected before human illness emerges, at which time preventive measures can be enacted. Migrating birds, commercially shipped livestock, and domesticated and wild animals targeted in the pet trade could play a role in spreading and maintaining an epidemic over large distances. Transportation of zoonotic agents by means of infected personnel, animals or inanimate objects (e.g., vehicles, food, souvenirs) from an operation theater to the home country (or vice versa) could generate an outbreak.

II. OBJECTIVES

- Defining priority zoonotic and vector-borne pathogens of importance to NATO operations.
- Identification and surveillance of reservoirs and vectors of the priority pathogens.
- Listing of fast, reliable detection and identification methods for relevant viral, bacterial, parasitic and other zoonoses and vector-borne diseases.
- Defining the necessary and standardized methods, materials and equipment for the sampling, handling, detection, and identification of reservoirs, vectors and pathogens.
- Gap analysis to identify shortcomings in knowledge and capabilities regarding zoonotic and vector-borne diseases.
- Organization of a specialist conference (4th year of RTG).

III. RESOURCES**A. Membership**

RTG Members should be veterinarians or infectious diseases specialists (military and/or civilian) with experience in diagnosis and/or epidemiology. Technicians and field workers with experience in diagnostic procedures and/or fieldwork and/or surveillance techniques are also invited. PfP, MD- and Contact countries are invited to participate.

B. Technical Team Leader and Leading Nation

Technical Team Leader: Med Lt Col C. Vandenvelde (BEL), Mr. P. Heyman (BEL).

Leading Nation: Belgium (BEL).

C. National and/or NATO Resources Needed

Funding for travel and other expenses of the technical team members is provided by the member's nation.

D. CSO Resources Needed

Consultant funding may be needed. Funding for members from PfP, MD and/or contact countries from the STO cooperation program may be needed.

IV. SECURITY CLASSIFICATION LEVEL

No security restrictions, TR and work will have the security status "Public Release".

V. PARTICIPATION BY PARTNER NATIONS

All countries from PfP-Nations, MD-Nations, Istanbul Cooperation Initiative and Contact Nations are invited to participate.

VI. LIAISON**COMEDS**

- BioMEDAC
- FHP expert panel
- Veterinary expert panel
- Medical Intelligence

COE, Budapest
DHSC

***Note:** These TORs are the official contract between all Nations which will be involved in the activity. As such, and contrary to the TAP, it should be as detailed and as long as necessary to secure the good management and success of the activity.*

STO TOR FORM – May 2012



Annex C – PROGRAM OF WORK (POW)

C.1 MAJOR ITEMS OF WORK

- Defining priority zoonotic and vector-borne pathogens of importance to NATO operations.
- Identification and surveillance of reservoirs and vectors of the priority pathogens.
- Listing of fast, reliable detection and identification methods for relevant viral, bacterial, parasitic and other zoonoses and vector-borne diseases.
- Defining the necessary and standardized methods, materials and equipment for the sampling, handling, detection, and identification of reservoirs, vectors and pathogens.
- Gap analysis to identify shortcomings in knowledge and capabilities regarding zoonotic and vector-borne diseases.

C.2 TIME SCALE

- Defining priority zoonotic and vector-borne pathogens of importance to NATO operations.
 - *Start 2012 – end 2014.*
- Identification and surveillance of reservoirs and vectors of the priority pathogens.
 - *Start 2012 – end 2014.*
- Listing of fast, reliable detection and identification methods for relevant viral, bacterial, parasitic and other zoonoses and vector-borne diseases.
 - *Start 2012 – end 2014.*
- Defining the necessary and standardized methods, materials and equipment for the sampling, handling, detection, and identification of reservoirs, vectors and pathogens.
 - *2013: consultation of NATO.*
 - *2014: report.*
- On-sight surveillance of priority pathogens.
 - *2012 – 2013: organization of sampling missions in collaboration with participating nations.*
 - *2014: recommendations report.*
- Gap analysis to identify shortcomings in knowledge and capabilities regarding zoonotic and vector-borne diseases.
 - *2015: Final report.*

C.3 MILESTONES

- Defining priority zoonotic and vector-borne pathogens of importance to NATO operations.
 - *Semi-annual meeting and annual progress report.*
- Listing of fast, reliable detection and identification methods for relevant viral, bacterial, parasitic and other zoonoses and vector-borne diseases.
 - *Annual progress report.*
- Defining the necessary and standardized methods, materials and equipment for the sampling, handling, detection, and identification of reservoirs, vectors and pathogens.
 - *Report after completion of each section (viral, bacterial, and parasitic).*
 - *2014: recommendations report.*
- Gap analysis to identify shortcomings in knowledge and capabilities regarding zoonotic and vector-borne diseases.
 - *2015: Final report.*

C.4 PARTICIPATING NATIONS

BEL, CZE, DEU, FRA, ITA, ROU, SVN, TUR, USA.

Other NATO nations, non-NATO nations, PfP nations, Mediterranean Dialogue nations and Contact Nations which are willing to participate are kindly invited to contact the Technical Team Leaders.

C.5 NATIONAL/AGENCY CONTRIBUTIONS

- Manpower: Individual nation's resources will be required.
- Individual nation's resources will be required to support RTG members in data acquisition.
- Individual nation's resources will be required to support RTG members in organizing surveillance.

C.6 HARDWARE/SOFTWARE

- Hardware supplied by the MoD-IT Depts of the respective participating nations.
- Software packages (e.g., Microsoft Office) supplied by the MoD-IT Depts of the respective participating nations.

C.7 PARTICIPANTS

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STO POW FORM – May 2012

Annex D – PATHOGEN SAFETY DATA SHEET *ANAPLASMA*

D.1 GENUS *ANAPLASMA* (*A. PHAGOCYTOPHILUM*, *A. PLATYS*, *A. MARGINALE*, *A. BOVIS*, *A. OVIS*)

D.1.1 Pathogen Safety Data Sheet – Infectious Substances

D.1.1.1 Infectious Agent

NAME: Anaplasma spp. (A. phagocytophilum, A. platys, A. marginale, A. bovis, A. ovis).

SYNONYM OR CROSS REFERENCE: N/A.

CHARACTERISTICS: Anaplasma is small (0,4 – 1,5 µm) pleomorphic, nonmotile Gram negative, obligate intracellular bacterium, occurs singly or in compact inclusions. *A. phagocytophilum* is a very heterogeneous organism, and genetic variants can differ in their virulence for host species.

D.1.1.2 Health Hazard

PATHOGENICITY: The consequences of exposure vary from asymptomatic infections or mild symptoms to a severe, potentially fatal illness. The disease is characterized by the acute onset of nonspecific febrile illness. Typical symptoms include fever, headache, fatigue, and muscle aches. It is often accompanied with thrombocytopenia, leukopenia and elevated levels of hepatic enzymes in the blood.

The disease is known as Human Granulocytic Anaplasmosis (HGA) in people, canine granulocytic anaplasmosis in dogs, equine granulocytic anaplasmosis in horses, and tick-borne fever in ruminants. *Anaplasma* species, of unknown pathogenicity for humans and animals, have been identified in ticks worldwide. The isolates of *A. phagocytophilum* maintained in some animal reservoir hosts might not affect people. The remaining *Anaplasma* species are not thought to be zoonotic.

EPIDEMIOLOGY: Bacterium is widespread in nature. It is transmitted to humans by the bite of an infected tick. *A. phagocytophilum* seems to be especially heterogeneous. Although this organism affects humans, horses, dogs and cats in both Eastern and Western Hemispheres, the variants found in the Americas do not seem to cause illness in ruminants.

In the early 1990s, patients with a tick bite history experiencing a febrile illness similar to human monocytotropic ehrlichiosis. The cases were distinguishable by the presence of inclusion bodies in granulocytes rather than monocytes. More than 2900 cases have been reported to CDC between 1994 and 2005.

HOST RANGE: A. phagocytophilum infects humans and many species of animals.

INFECTIOUS DOSE: Unknown.

MODE OF TRANSMISSION: Transmission by ticks in the family *Ixodidae*. They are usually maintained in cycles between ticks and wild or domesticated animal reservoir hosts, which can sometimes remain infected for long periods.

ANNEX D – PATHOGEN SAFETY DATA SHEET ANAPLASMA

A. phagocytophilum is transmitted by *Ixodes* species:

- *I. ricinus* – the major vector in Europe;
- *I. persulcatus* – the major vector in Asia; and
- *I. scapularis* and *I. pacificus* – the major vectors in North America.

This organism can also be found in other tick vectors: *I. trianguliceps*, *I. spinipalpis*.

Genetic variants of *A. phagocytophilum* might be adapted to different reservoir hosts and/or ticks species and maintained in distinct cycles.

Transstadial transmission is thought to be important in the life cycles of *Ehrlichia spp.* Transovarial transmission has never been shown and is not thought to occur.

Perinatal transmission has been reported. It may occasionally be transmitted by other routes, especially in medical procedures that transfer blood, bone marrow or solid organs. Transmission on contaminated needles and mechanical transmission by biting insects are also theoretically possible. Transmission by close, direct contact with blood and respiratory secretions are needed to confirm.

INCUBATION PERIOD: From 5 to 21 days. The U.S. Centers for Disease Control and Prevention (CDC) estimates that symptoms typically appear in 1 – 2 weeks.

COMMUNICABILITY: The possibility of person-to-person transmission by close, direct contact with blood and body secretions remains to be confirmed.

D.1.1.3 Dissemination

RESERVOIR: The reservoir hosts include numerous wild and domestic animals, primarily small mammals. Humans serve as dead-end hosts.

ZOONOSIS: Yes.

VECTORS: Transmitted to humans by the bite of an infected tick. Mechanical transmission by biting insects is also theoretically possible.

D.1.1.4 Viability

DRUG SUSCEPTIBILITY: Early treatment is critical. Usually treated with tetracyclines; doxycycline is currently the drug of choice in people. Other antibiotics are occasionally employed (contraindications to the use of tetracyclines). Rifampin has been used successfully in some pregnant women. Chloramphenicol is not recommended.

DRUG RESISTANCE: Resistance to doxycycline has not been documented.

SUSCEPTIBILITY TO DISINFECTANTS: There is little or no research on the disinfectant susceptibility of these obligate intracellular pathogens.

PHYSICAL INACTIVATION: Susceptible to moist heat (121°C for at least 15 min) and dry heat (160 – 170 °C for at least 1 hour).

SURVIVAL OUTSIDE HOST: N/A.

D.1.1.5 Medical

SURVEILLANCE: Monitor for symptoms; serology (indirect immunofluorescence assay, enzyme-linked immunosorbent assays); microscopic examination for the detection of intracytoplasmic inclusions (morulae) in leucocytes; polymerase chain reaction; cultivation.

Cross-reactions between bacteria in the genera *Anaplasma*, *Ehrlichia* and *Neorickettsia* can complicate definitive identification. There is significant cross-reactivity between *E. ewingii* and *E. chaffensis*.

FIRST AID / TREATMENT: Antibiotic therapy.

IMMUNIZATION: Vaccine is not available for use in humans.

PROPHYLAXIS: Avoidance of tick bites (protective clothing and footwear, tick repellents). Prophylactic antibiotic therapy is not recommended after a tick bite.

D.1.1.6 Laboratory Hazards

LABORATORY-ACQUIRED INFECTIONS: No information.

SOURCES/SPECIMENS: Cultures, blood, tissues, ticks.

PRIMARY HAZARDS: Direct contact of skin or mucous membranes with infectious materials, accidental parenteral inoculation, ingestion, exposure to aerosols and infectious droplets.

SPECIAL HAZARDS: None.

D.1.1.7 Recommended Precautions

CONTAINMENT REQUIREMENTS: Biosafety level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials, animals, or cultures.

PROTECTIVE CLOTHING: Laboratory coat; gloves in case of direct contact with infectious materials; eye protection for work with infectious material.

OTHER PRECAUTIONS: All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet. The use of needles, syringes, and other sharp objects should be strictly limited. Additional precautions should be considered with work involving animals.

D.1.1.8 Handling Information

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towels and apply an appropriate disinfectant, starting at perimeter and working towards the center; allow sufficient contact time before clean up.

DISPOSAL: Decontaminate before disposal; steam sterilization, incineration, chemical disinfection.

STORAGE: In sealed containers that are appropriately labelled.

D.1.1.9 Miscellaneous Information

DATE PREPARED: June 2015.

PREPARED BY: NATO RTG HFM-230 Detection and Identification Methods for Zoonotic and Vector-Borne Agents.

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Annex E – PATHOGEN SAFETY DATA SHEET *ERLICHIA*

E.1 GENUS *ERLICHIA* (*E. CANIS*, *E. CHAFFEENSIS*, *E. EWINGII*, *E. MURIS*, *E. RUMINANTII*)

E.1.1 Pathogen Safety Data Sheet – Infectious Substances

E.1.1.1 Infectious Agent

NAME: Erlichia spp. (E. chaffeensis, E. ewingii, E. muris, E. canis, E. ruminantium).

SYNONYM OR CROSS REFERENCE: N/A.

CHARACTERISTICS: Erlichia is small (0,4 – 1,5 µm) pleomorphic, nonmotile Gram negative, obligate intracellular bacterium, occurs singly or in compact inclusions.

E.1.1.2 Health Hazard

PATHOGENICITY: The consequences of exposure vary from asymptomatic infections or mild symptoms to a severe, potentially fatal illness. The disease is characterized by the acute onset of nonspecific febrile illness. Typical symptoms include fever, headache, fatigue, and muscle aches. It is often accompanied with thrombocytopenia, leukopenia and elevated levels of hepatic enzymes in the blood.

E. chaffeensis infects monocytes, causing illness in both dog and people. In people, this disease is called Human Monocytic Erlichiosis (HME). *E. ewingii* infects granulocytes and is zoonotic. The illness is sometimes known as canine granulocytic erlichiosis in dogs. However, Human Ewingii Erichiosis (HEE) is preferred name for the disease in people. *E. muris* infects monocytes in rodents, it might also be zoonotic. *E. canis* causes Canine Monocytic Erlichiosis (CME). This organism has been implicated rarely in human illness. *E. ruminantium* is the agent of heartwater in ruminants. It is not thought to be zoonotic, but a report describing several infected people exists.

EPIDEMIOLOGY: Bacterium is widespread in nature. It is transmitted to humans by the bite of an infected tick. *E. chaffeensis* was originally described from North America, but it was detected in parts of South America, Asia and Africa. *E. ewingii* occurs mainly in North America, it was recently detected in South America and Africa (Cameroon). *E. muris* has been found in ticks from Eastern Europe to Japan. A closely related organism was recently described in human patients in North America. *E. canis* occurs worldwide, distribution within an area varies with the presence and density of tick vectors.

HME was first described in 1986, more than 2300 cases have been reported to the CDC during 19 years.

HOST RANGE: Humans, dogs, rodents, ruminants.

INFECTIOUS DOSE: Unknown.

MODE OF TRANSMISSION: Transmission by ticks in the family *Ixodidae*. They are usually maintained in cycles between ticks and wild or domesticated animal reservoir hosts, which can sometimes remain infected for long periods.

ANNEX E – PATHOGEN SAFETY DATA SHEET *ERLICHIA*

E. canis: Rhipicephalus sanguineus is the primary vector, it can also be transmitted experimentally by *Dermatocentor variabilis*, other secondary vectors (*Amblyomma cajennense*) have been suggested.

E. chaffeensis: *Amblyomma americanum* is the most important vector in North America. Secondary vectors *D. variabilis* and *R. sanguineus* might have a minor role. Outside North America has been found in ticks in the genera *Amblyomma*, *Haemaphysalis*, *Dermatocentor* and *Ixodes* in Asia, in *R. sanguineus* in Cameroon, and in *Am. parvum* in Argentina.

E. ewingii: *Amblyomma americanum* is the most important vector in North America.

E. muris: Can be transmitted by *Haemaphysalis flava* and *Ixodes persulcatus* complex ticks species.

Transstadial transmission is thought to be important in the life cycles of *Erlichia spp.* Transovarial transmission has never been shown and is not thought to occur.

Perinatal transmission has been reported. It may occasionally be transmitted by other routes, especially in medical procedures that transfer blood, bone marrow or solid organs. Transmission on contaminated needles and mechanical transmission by biting insects are also theoretically possible. Transmission by close, direct contact with blood and respiratory secretions are needed to confirm.

INCUBATION PERIOD: From 5 to 21 days. The U.S. Centers for Disease Control and Prevention (CDC) estimates that symptoms typically appear in 1 – 2 weeks.

COMMUNICABILITY: The possibility of person-to-person transmission by close, direct contact with blood and body secretions remains to be confirmed.

E.1.1.3 Dissemination

RESERVOIR: The reservoir hosts include numerous wild and domestic animals.

ZOONOSIS: Yes.

VECTORS: Transmitted to humans by the bite of an infected tick. Mechanical transmission by biting insects is also theoretically possible.

E.1.1.4 Viability

DRUG SUSCEPTIBILITY: Early treatment is critical. Usually treated with tetracyclines; doxycycline is currently the drug of choice in people. Other antibiotics are occasionally employed (contraindications to the use of tetracyclines). Rifampin has been used successfully in some pregnant women. Chloramphenicol is not recommended.

DRUG RESISTANCE: Resistance to doxycycline has not been documented.

SUSCEPTIBILITY TO DISINFECTANTS: There is little or no research on the disinfectant susceptibility of these obligate intracellular pathogens.

PHYSICAL INACTIVATION: Susceptible to moist heat (121°C for at least 15 min) and dry heat (160 – 170 °C for at least 1 hour).

SURVIVAL OUTSIDE HOST: Some *Ehrlichia* spp. can remain viable more than a week in preserved, refrigerated whole blood.

E.1.1.5 Medical

SURVEILLANCE: Monitor for symptoms; serology (indirect immunofluorescence assay, enzyme-linked immunosorbent assays); microscopic examination for the detection of intracytoplasmic inclusions (morulae) in leucocytes; polymerase chain reaction; cultivation.

Cross-reactions between bacteria in the genera *Anaplasma*, *Ehrlichia* and *Neorickettsia* can complicate definitive identification. There is significant cross-reactivity between *E. ewingii* and *E. chaffeensis*.

FIRST AID / TREATMENT: Antibiotic therapy.

IMMUNIZATION: Vaccine is not available for use in humans.

PROPHYLAXIS: Avoidance of tick bites (protective clothing and footwear, tick repellents). Prophylactic antibiotic therapy is not recommended after a tick bite.

E.1.1.6 Laboratory Hazards

LABORATORY-ACQUIRED INFECTIONS: No information.

SOURCES/SPECIMENS: Cultures, blood, tissues, ticks.

PRIMARY HAZARDS: Direct contact of skin or mucous membranes with infectious materials, accidental parenteral inoculation, ingestion, exposure to aerosols and infectious droplets.

SPECIAL HAZARDS: None.

E.1.1.7 Recommended Precautions

CONTAINMENT REQUIREMENTS: Biosafety level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials, animals, or cultures.

PROTECTIVE CLOTHING: Laboratory coat; gloves in case of direct contact with infectious materials; eye protection for work with infectious material.

OTHER PRECAUTIONS: All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet. The use of needles, syringes, and other sharp objects should be strictly limited. Additional precautions should be considered with work involving animals.

E.1.1.8 Handling Information

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towels and apply an appropriate disinfectant, starting at perimeter and working towards the center; allow sufficient contact time before clean up.

DISPOSAL: Decontaminate before disposal; steam sterilization, incineration, chemical disinfection.

ANNEX E – PATHOGEN SAFETY DATA SHEET *ERLICHIA*

STORAGE: In sealed containers that are appropriately labelled.

E.1.1.9 Miscellaneous Information

DATE PREPARED: June 2015.

PREPARED BY: NATO RTG HFM-230 Detection and Identification Methods for Zoonotic and Vector-borne Agents.

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E.2 SOURCES

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Annex F – PATHOGEN SAFETY DATA SHEET *STREPTOCOCCUS SUIIS*

F.1 *STREPTOCOCCUS SUIIS*

F.1.1 Pathogen Safety Data Sheet – Infectious Substances

F.1.1.1 Infectious Agent

NAME: Streptococcus suis (S. suis).

SYNONYM OR CROSS REFERENCE: Streptococcal sore throat, septic sore throat.

CHARACTERISTICS: *Streptococcus suis* is a gram-positive coccus that is frequently seen in pairs but can also be single or in short chains. The 35 *S. suis* serotypes can be identified by agglutination.

F.1.1.2 Hazard Identification

PATHOGENICITY/TOXICITY: *S. suis* causes a systemic infection in humans that affects several organ systems; meningitis is the most common clinical manifestation. The presenting features of *S. suis* meningitis are generally similar to those of other bacterial pyogenic meningitis and include headache, fever, vomiting, and meningeal signs. The duration of illness before hospital admission was 2–5 days. One striking feature is subjective hearing loss, which may be reported by up to one-half of patients at presentation or a few days later. Some patients improve over time, but others do not. Up to 30% of the patients also have skin findings, including petechiae, and purpura, all of which can be extensive, and hemorrhagic bullae. Gangrene of the fingers and toes may also be seen in a minority of patients at a later stage in the disease. Less common manifestations of *S. suis* infection include acute and subacute endocarditis, acute pyogenic arthritis, endophthalmitis and uveitis, and epidural abscess. Meningitis is often accompanied by bacteremia, similar to *Streptococcus pneumoniae* and *Neisseria meningitidis* meningitis. *S. suis* infection can be complicated by acute renal failure requiring renal replacement therapy, acute respiratory distress syndrome necessitating ventilation, and consumptive coagulopathy. Outbreaks of *S. suis* infection in China have highlighted the importance and relative high frequency of a severe sepsis syndrome – with some features suggestive of toxic shock syndrome – associated with a high mortality.

EPIDEMIOLOGY: *S. suis* infection in pigs is reported worldwide, from North to South America (Brazil), Europe, Asia (China, Thailand, Vietnam, and Japan), Australia, and New Zealand. Healthy pigs can carry multiple serotypes of *S. suis* in their nasal cavities, tonsils, and upper respiratory, genital, and alimentary tracts. Of the 35 known serotypes, only a limited number are responsible for infections in pigs, including serotypes 1 – 9 and 14. Serotype 2 is considered to be the most pathogenic for both humans and pigs. Human *S. suis* infections are most often reported from countries where pig-rearing is common. The relative high mean patient age (4 – 55 years) and almost complete absence of children in case series, as well as the high male-to-female patient ratio.

HOST RANGE: *Streptococcus suis* has been found in humans. In addition to pigs, *S. suis* can be isolated from other animals, such as ruminants, cats, dogs, deer, and horses, and is believed to be a commensal in the respiratory tract and the intestinal flora.

INFECTIOUS DOSE: The infectious dose is about 10^8 bacteria in wounds and skin lesions (in animal experiments).

ANNEX F – PATHOGEN SAFETY DATA SHEET *STREPTOCOCCUS SUIIS*

MODE OF TRANSMISSION: In pigs *S. suis* is usually transmitted nasally or orally and colonizes the palatine tonsils of both clinically ill and healthy pigs. The infant piglets become infected after contact with colonized sows. Rates of asymptomatic carriage may be as high as 80%. In Asia farmers often share accommodations with pigs, and it is common practice for diseased animals to be slaughtered at home and consumed.

INCUBATION PERIOD: The incubation period for this bacterium is between 3 – 14 days.

COMMUNICABILITY: Although rare, the disease can be spread from human-to-human.

F.1.1.3 Dissemination

RESERVOIR: Pigs are the most important reservoir host although the bacterium has been found in several farm animals and pets.

ZOONOSIS: The disease can be spread from animals to humans via blood and meat in slaughtering and by food sources (contaminated pork).

VECTORS: None.

F.1.1.4 Stability and Viability

DRUG SUSCEPTIBILITY: Similar to the protocol for any other patient with suspected bacterial meningitis in *Streptococcus suis* infections, antibiotic treatment should be started without delay. *S. suis* is susceptible to penicillin, ceftriaxone, and vancomycin. Resistance was seen to tetracyclin, erythromycin and cloramphenicol.

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 2 – 5 % phenol, 1% sodium hypochlorite, 70% ethanol, 4% formaldehyde, 2% glutaraldehyde, 2% peracetic acid, 3 – 6 % hydrogen peroxide and 0.16% iodine.

PHYSICAL INACTIVATION: Bacteria are sensitive to moist heat (121°C for at least 12 minutes) and dry heat (170°C for 1 hour).

SURVIVAL OUTSIDE HOST: The bacterium is stable in meat, blood and animal excretions.

F.1.1.5 First Aid / Medical

SURVEILLANCE: Monitor for symptoms. The bacterium can be isolated in stool, tissue samples, blood, CSF and pus. PCR and ELISA can also be used to diagnose the disease. Determination of *S. suis* to the species level can be performed with biochemical tests. Commercial systems (e.g., API Strep; Biomerieux) can also be used. The 35 *S. suis* serotypes can be identified by agglutination with a panel of antiserum samples. Serotyping is performed in reference laboratories. Although *S. suis* can be cultured from CSF or blood samples with use of standard microbiological techniques, it is often misidentified, or infection goes undiagnosed. Molecular techniques have improved the detection. In regions with pig farming industries one should consider *S. suis* if optochin-resistant streptococci are cultured from a CSF sample obtained from a patient with meningitis.

Note: All diagnostic methods are not necessarily available in all countries.

FIRST AID / TREATMENT: Similar to the protocol for any other patient with suspected bacterial meningitis, in *Streptococcus suis* infections antibiotic treatment should be started without delay. The use of dexamethasone as an adjunctive treatment to reduce mortality and improve the outcome of bacterial meningitis remains controversial.

IMMUNIZATION: None.

PROPHYLAXIS: Preventive measures, such as wearing gloves during processing pig meat or slaughtering, hand washing after handling raw pork meat, and thorough cooking of pork, should prevent the majority of cases. Travelers should be aware that dietary habits in some countries may pose a risk for infectious diseases, including *S. suis* infection.

F.1.1.6 Laboratory Hazards

LABORATORY-ACQUIRED INFECTIONS: None.

SOURCES/SPECIMENS: The bacterium can be found in blood, meat, stool, tonsils or lymph node tissues.

PRIMARY HAZARDS: Accidental parenteral inoculation is always a risk when working with pathogens and ingestion of the infectious agent (via contaminated hands) is a hazard for laboratory personnel working with pathogens.

SPECIAL HAZARDS: Contact with infected animals can be a risk.

F.1.1.7 Exposure Controls / Personal Protection

RISK GROUP CLASSIFICATION: Risk group 2.

CONTAINMENT REQUIREMENTS: Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials, animals, or cultures.

PROTECTIVE CLOTHING: Lab coat. Gloves when direct skin contact with infected materials or animals is unavoidable. Eye protection must be used where there is a known or potential risk of exposure to splashes.

OTHER PRECAUTIONS: All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC). The use of needles, syringes, and other sharp objects should be strictly limited. Additional precautions should be considered with work involving animals or large scale activities.

F.1.1.8 Handling and Storage

SPILLS: Allow aerosols to settle and, wearing protective clothing, gently cover spill with paper towels and apply an appropriate disinfectant, starting at the perimeter and working towards the centre. Allow sufficient contact time before clean up.

DISPOSAL: Decontaminate all wastes that contain or have come in contact with the infectious organism by autoclave, chemical disinfection, gamma irradiation, or incineration before disposing.

STORAGE: The infectious agent should be stored in leak-proof containers that are appropriately labelled.

F.1.1.9 Regulatory and Other Information

REGULATORY INFORMATION: The import, transport, and use of pathogens in Europe is regulated under many regulatory bodies, including the Public Health Agencies. Users are responsible for ensuring they are compliant with all relevant acts, regulations, guidelines, and standards.

UPDATED: August 2014.

PREPARED BY: NATO RTG HFM-230 Working Group: Development of Depository of fast and reliable Detection Methods for Zoonotic Agents.

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NATO RTG HFM-230 Working Group, 2014, France.

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14. Abstract			
In the context of One Health, interest in new and (re)emerging zoonotic and vector-borne diseases is increasing among the interdisciplinary fields of human and veterinary medicine. The potential of zoonotic and vector-borne diseases to impact the mission or to have increased virulence in populations lacking immunity make them of significant concern to military forces. Infectious diseases acquired in the operation zone continue to account for more military clinical encounters than battlefield injuries. In addition, distinguishing naturally occurring outbreaks of zoonotic and vector-borne diseases from intentional biologic attack is of concern. Concerted action for detection and surveillance, and subsequent dissemination of data and information between NATO partners is thus of importance. Moreover, decision makers are in need of tools to prioritize the pathogens of importance to military operations. A NATO working group to address these issues was established in 2012. Members from Belgium, the Czech Republic, France, Germany, Italy, Romania, Slovenia, Turkey and the United States worked together until Spring 2016 to identify priority zoonotic and vector-borne pathogens, and to compile a consolidated list of fast and reliable detection methods for each of these pathogens, divided into 'first line methods' not needing a high-tech environment and 'second line methods'. This report includes the products developed by the group as well as a discussion of the findings, knowledge gaps and a recommended way forward.			





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