



Defence Research and  
Development Canada

Recherche et développement  
pour la défense Canada



# **Feasibility Study of Canadian Culture Collections**

*Final Report*

D.J. Netolitzky

Contract Scientific Authority: B. Kournikakis  
DRDC Suffield

The scientific or technical validity of this Contract Report is entirely the responsibility of the contractor and the contents do not necessarily have the approval or endorsement of Defence R&D Canada.

Contract Report  
DRDC Suffield CR 2003-133  
September 2003

Canada



# **Feasibility Study of Canadian Culture Collections**

*Final Report*

D.J. Netolitzky

Dr Donald J. Netolitzky  
7307 106 Street  
Edmonton AB T6E 4V7

Contract Number: W7702-03-P159/001/EDM

Contract Scientific Authorities: B. Kournikakis, 403-544-4631

The scientific or technical validity of this Contract Report is entirely the responsibility of the contractor and the contents do not necessarily have the approval or endorsement of Defence R&D Canada.

## **Defence R&D Canada – Suffield**

Contract Report

DRDC Suffield CR 2003-133

September 2003

© Her Majesty the Queen as represented by the Minister of National Defence, 2003

© Sa majesté la reine, représentée par le ministre de la Défense nationale, 2003

# Final Report : Feasibility Study of Canadian Pathogen Culture Collections

Sept. 5, 2003

## Author

Dr. Donald J. Netolitzky, PhD  
7307 106 St. Edmonton, Alberta, Canada  
T6E 4V7  
(780) 433-6446  
Verlaag@telus.net

## Technical Authority

Dr. Bill Kournikakis  
Department of National Defence  
Defence R&D Canada - Suffield  
P.O. Box 4000  
Medicine Hat, Alberta, Canada  
T1A 8K6

## Contract

Feasibility of Establishing a Canadian Type Culture Collection,  
Contract Number W7702-03P159/001/EDM  
June 2 – Sept 5, 2003

This document is subject to copyright, printed, electronic, or and other form or medium  
by:

HER MAJESTY THE QUEEN IN RIGHT OF CANADA (2003)

or

SA MAJESTE LA REINE DU CHEF DU CANADA (2003)

## Table of Contents

<u>Report Sections</u>	page number
I Introduction	3
II Key Collection Features	6
III Collection Holding Categories	10
IV Project Scale / Expansion / Organization	14
V Pathogen Lists and Analysis	22
VI Collection Procedures	30
VII Manpower	38
VIII Capital Requirements	44
IX Ongoing Requirements	48
X Database / IT	51
XI Culture Sources and Costs	56
XII Total Costs and Implications	59
XIII Conclusions	61
<u>References</u>	64
<u>Appendices</u>	
I – BW Agent Lists	67
II – Culture Collection Procedure References	70
III – Web Collection Search Engine Sample and Sample Output	71
IV – Database Item / Entry Outline	73
V - Culture Collection Training Sources	82
VI – Culture Collection Strain Holdings / Staff	83
VII – BL 3/4 and BW Agent List	84
VIII – Pathogenic Bacteria	88
IX – Pathogenic Viruses	95
X – Pathogenic Fungi	100
XI – Sample Equipment and Suppliers	104
XII - Detailed Equipment Cost Breakdown	106
XIII – Key Culture Collections used During Survey	110
XIV – Possible Sample Sources	111

## I - Introduction

Canada has never established a national type culture collection, but instead has depended upon American resources, primarily the American Type Culture Collection (ATCC). Since the September 11, 2001 terrorist attacks, American sources for pathogenic organisms have all but closed to legitimate Canadian medical and governmental requests for samples. This sudden restriction only promises to become more severe as time passes and other governments follow suit. Should this situation persist, Canada may literally find itself unable to access the very biological resources required for basic research, accurate diagnosis of disease, and identification of biological warfare (BW) threats. Therefore, a window of opportunity exists to obtain biological material from non-American sources, and establish indigenous Canadian collections. This report investigates the feasibility of exploiting this opportunity and creating a Canadian pathogen-oriented type culture collection.

A type culture collection is an assortment of biological specimens, maintained and preserved in a viable condition for future use. Collections range from the very specialized, containing strains of a single species, to large national repositories including all kinds of organisms. Culture collections are not static, but grow and develop over time depending on the needs of those they serve. While the goals and contents of a collection may change, every effort is made to ensure the organisms within the collection remain unaltered, typically in a stored, inert condition. Thus, a culture collection is, in effect, a biological library in which important information can be stored and retrieved whenever needed.

Culture collections are valuable to government, academic research, and industry in many ways.

- 1) Medical Applications : culture collections can provide known specimens for identification purposes, material for medical research, and raw materials for vaccines. As pathogens are discovered and analyzed, they may be safely and securely deposited in a culture collection for future use.
- 2) Biological Warfare Defence : BW defence has needs similar to those found in the medical community.
- 3) Agriculture : private and public agriculture labs require known samples for identification purposes. A collection is particularly useful when identifying and excluding rare or foreign pathogens, a significant issue when examining imported foodstuffs, animals, and plants.
- 4) Science : in addition to serving as a source for research material, culture collections are also valuable in storing characterized organisms. Much biological research is analysis of the properties and operation of living things, quite literally dissecting how these organisms work. Should a well-analyzed organism or strain be lost or discarded, the probability of recovering that exact strain from the environment is effectively nil. As a consequence, further research on a now 'lost' strain is effectively impossible. Culture collections can act as repositories for these organisms,

preserving a key research resource against accidental loss, or a switch in a researcher's priorities. In this sense, culture collections function in a manner analogous to published journals, preserving scientific data in a living form for future generations.

- 5) Industry : industry also requires organisms with known and unusual biological properties such as unique metabolic pathways, antigenic properties, or growth conditions (eg. thermophiles). Culture collections are an ideal source for organisms with such properties, provided strains are sufficiently well documented.

As culture collections are expert in preserving biological materials, many collections offer small scale and / or bulk preservation of microorganisms as a commercial service. Culture collections may also act as safe commercial repositories of industrial biological material (see for example ATCC (1) and UKNCC (2)).

Storing organisms in culture collections is a required step in filing a biological patent. An international standard for deposition of patented organisms has been developed: the Budapest Treaty on the International Recognition of the Deposit of Micro-Organisms for the Purposes of Patent Procedure (3) (see also (4)). Most industrialized countries have signed this treaty, and established national 'patent' collections (International Depositary Authorities (IDA)). Canada has not established a dedicated IDA, though the National Microbiology Laboratory (NML) provides this service on a semi-formal basis.

- 6) Biodiversity : the natural value of genetic resources has increasingly met world-wide acceptance, particularly as expressed in the United Nation's Convention on Biodiversity (5). Simply, different organisms encode unique biological traits within their genetic material. The extinction of any species, strain, or subpopulation therefore represents the elimination of a potential resource. This fact has become particularly apparent in agriculture, where the use of genetically identical monocultures has led to the loss of many unusual biological characteristics in both plants (6) and animals (7). Culture collections maintain unique organisms, help maximize biodiversity, and preserve genetic variations.

- 7) Education and Research : culture collection staff become expert in a number of valuable scientific techniques; organism identification, growing unusual organisms (eg. extremophiles (8)), and techniques for preservation, storage and maintenance of biological resources (see for example UKNCC (9)). This expertise allows culture collections to provide training, either in formal courses or as consultants. Culture collections are also the ideal site to develop new related techniques and technologies (eg. preservation of difficult organisms (10)).

- 8) Identification : as conclusive identification of microbial isolates is a key function of any culture collection, culture collections must develop expertise in identifying unknown organisms. In fact, a culture collection curator routinely encounters a greater number and variety of organisms than any other kind of biological or medical specialist. This expertise is itself a powerful tool when confronted with an unknown organism. Consequentially, many culture collections offer organism identification /



taxonomy services. In this sense, a well established culture collection is not merely a repository of biological materials, but also an active agent assisting medical, scientific, industrial and governmental entities when attempting to identify unknown organisms.

## **Medical and BW Specific Issues**

The culture collection proposed and described in this report is principally oriented, initially, towards medical applications and BW defence. Consequentially, a more detailed discussion of issues related to these two domains is appropriate.

### **1) Unknown Pathogen Identification**

Identifying an isolated but unidentified pathogen is a crucial task for both medicine and BW defence. Both need to rapidly and accurately identify microorganisms if the appropriate countermeasures are to be made. Culture collections provide comparison organisms to assist in the identification of unknown samples. Comparison strains need not be identical to an unknown to be useful, as was well illustrated during the recent appearance and spread of the SARS virus (the National Medical Laboratory's SARS Coronavirus identification (11) and the determination of the SARS genome sequence (12)). Comparison with previously identified strains may help determine the source of an unknown sample. For example, known strains clarified the BW agent used in the US anthrax letter terror attacks as a specific medical source strain.

While many medical labs retain 'known' samples, these facilities are not ideally suited to maintain these comparison strains. Growth in non-selective (laboratory) conditions results in genetic drift and the loss / alteration of key strain characteristics. A national repository for high quality medical type strains would be a significant resource for private and public medical diagnostic labs. This collection could establish a uniform 'national' standard for pathogen testing, especially if medical labs are required to periodically 'refresh' their comparison organisms from the national pathogen collection.

### **2) Access to Rare / Unusual Organisms**

Certain pathogens are not common in Canada, and are best stored in a specialized facility. Given the large number of known human pathogens, regional and private medical labs cannot practically maintain known type specimens of all pathogens. Some pathogens require special containment facilities which are extremely expensive to operate. A national pathogen collection can store and provide exotic and high threat pathogens. These rare pathogens need to be actively preserved, many particularly pathogenic / virulent strains and organisms periodically appear and then disappear, often for years. If these organisms are not artificially preserved, they will literally be unavailable for research, analysis, and comparison. Furthermore, international travel transfers what were once foreign and exotic pathogens into new regions, such as West Nile Virus into North America.

Many rare pathogens, such as the Ebola virus, cause extreme mortality and morbidity. Typically these pathogens are zoonoses and have not co-evolved with humans (13). While infrequently encountered medically, these organisms possess precisely the characteristics of ideal BW agents, and thus are likely to be encountered in the event of a

BW attack. Without a reliable source for these rare pathogens, BW defence research and reaction is significantly hampered.

### 3) Unconventional Pathogens

The medical and scientific community has become increasingly aware of the periodic appearance of previously unknown pathogens. These novel organisms may be created naturally by a variety of processes and the technology now exists to design man-made pathogens, presumably as BW agents.

Novel pathogens are generated naturally by processes such as recombination, and genetic transfer. Recombinant viruses are created by co-infection by two or more viruses, a process which is responsible for the continual generation of new Influenza Virus strains (14). DNA also moves between organisms (particularly prokaryotes) to a vastly larger degree than ever previously anticipated, a process known as lateral or horizontal transfer. Lateral DNA transfer accounts for the proliferation of antibiotic resistant bacteria (15), and also seems intimately associated with acquisition of pathogenic properties (15). Comparisons of benign and pathogenic bacterial genomes have revealed mobile DNA (plasmids) or recombinant genomic 'cassettes' carrying pathogenic markers (15). Therefore, DNA sequences in one species may later be found in a quite unrelated novel pathogen. Genetic exchange is crucial to the generation of new and dangerous organisms.

Engineered BW pathogens would likely be constructed in an analogous manner, adding foreign DNA sequences to produce characteristics such as antibiotic resistance, toxin production, or pathogenicity. Alternately, modifications could be intended to confuse and inhibit diagnosis: altering antigenic domains (also thereby evading vaccines), changing diagnostic metabolic characteristics, or modifying / eliminating characteristic nucleic acid sequences.

Understanding a novel pathogen is greatly assisted by data from characterized relatives. While some characteristics of an edited / recombinant organism may be altered, a more extensive comparison would likely reveal links to the original. As DNA moves naturally from one organism to another, the sequences which generate a novel pathogen could originate from practically any other species. As a consequence, culture collection holdings can greatly assist analysis of a hitherto unknown organism. Identifying organisms with common traits / motifs / genetic elements can lead to more rapid development of treatments and / or vaccines.

### 4) Vaccine Development

Vaccines are a crucial method of protection against medical pathogens and BW threats. Culture collections provide an excellent source of raw vaccine material, especially for unusual pathogens. Alternately, closely related non-pathogenic species may be suitable vaccine candidates, such as cowpox immunizing against smallpox and the recently proposed Kunjin Virus vaccine against West Nile virus (16).

## **What Kind of Collection is Useful for Medical / BW Applications**

While any stored organism has a medical / BW application, pathogens are naturally the most crucial holding in a medical / BW collection. Ideally, a collection

should have a representative of every organism to act as a 'type' species. Additional strains of the same pathogen are also useful, particularly if they possess unusual properties. In addition, close relatives of human pathogens are helpful, as these are the organisms most likely to cause confusion during identification.

Animal pathogens are also very valuable holdings, particularly in the case of viruses. While many pathogens are unable to exist in more than one species, some are capable of infecting a 'foreign' host. The results can be disastrous; these pathogens (zoonoses) often create the highest mortality and morbidity. Frequently, only a poor understanding exists of whether a particular animal pathogen species may also infect humans, and historically many 'new' pathogens are simply a new zoonosis, now introduced into man (eg. Hantavirus (17), HIV (18), SARS (11)). As a consequence, holding a wide diversity of animal, particularly mammalian pathogens, is helpful even if the pathogen has no known human symptoms. Viruses closely related to pathogenic human viruses are particularly significant threats.

Finally, any organism has its use. Our current appreciation of the extent of genetic exchange brings home the fact that the crucial DNA sequence characterizing a pathogen may have originated in a quite unlikely source. Predicting just which holding will prove invaluable is a difficult challenge, at best.

## II - Key Collection Features

For a type culture collection to be useful, the collection must fulfill four fundamental criteria:

- 1) the collection must be comprehensive and contain the organisms required by those accessing the collection.
- 2) the collection must be stable; the organisms stored must not change over time, and must remain viable.
- 3) the collection's strains must be accurate; whatever organisms are housed must be correctly identified, uncontaminated, and properly organized and catalogued.
- 4) the collection must be accessible; those using the collection must be able to obtain information describing strains as well as prompt sample delivery.

Each of these points deserves some additional analysis, especially in regards to a Canadian pathogen collection.

### 1) Comprehensive

Since the primary goal of the proposed collection is to provide a library of organisms which represent BW threats or are of medical significance, this report focuses on those groups. Ideally, the collection would include all known human pathogens, both local and exotic. Agricultural pathogens which do not affect humans are also suitable components of the collection, from a BW context. As most new human diseases are zoonoses (pathogens resident in species other than man), animal diseases are also of value, though are not addressed directly within this report.

A larger collection including organisms without significant pathogen potential is certainly a possibility, however planning such a collection is beyond the scope of this report, and would 'diffuse' the purpose of this collection. Nevertheless, additional microbiological resources are without a doubt useful, and may be incorporated within an larger culture collection strategy. This possibility is discussed in Section IV of the report (Project Scale and Organization).

### 2) Stable

For a collection strain to be useful, it must both remain viable, and also retain the characteristics it possessed when added to the collection. These two objectives are, unfortunately, mutually exclusive. When a strain is stored in a non-growing, 'inert' state, it gradually loses viability as individual organisms die. Different storage procedures can slow this loss of viability, but ultimately any culture will die. However, growing an organism allows it to change and mutate, ultimately producing a strain whose genetic and phenotypic characteristics differ from the original strain. In essence when grown in culture, an organism evolves to match the unnatural culture environment. This process is particularly true of pathogenic organisms. Growing a pathogen outside its natural host can very rapidly generate an 'attenuated' strain which no longer has the ability to cause disease.

Thus, a culture collection is caught between two mutually incompatible objectives. To remain viable, organisms must be grown, but that very growth process

allows mutation and evolutionary drift. Therefore, culture collection procedures must balance these two factors, attempting to keep organisms both alive and unchanged. The key to achieving this goal is selecting storage techniques which extend the period between culture re-growth.

A second significant culture collection issue is culture loss due to some mishap (a power or equipment failure). Ideally, a culture collection is at least partially duplicated and redundant. The duplicate collection, or at least a few samples should be stored in an off-site facility.

### 3) Accurate

The accuracy of a culture's holdings is a key issue. Organisms must be correctly identified, and stocks must be uncontaminated. Cultures must be physically organized to reduce risk of loss or confusion, and all samples must be carefully and exhaustively documented, principally using a computer database. Data cataloguing and physical organization is relatively uncomplicated, however correct specimen identification is not.

Correct identification of organisms is one of the principle tasks of a culture collection, and therefore collection curators and researchers need to be experts in taxonomy. A surprising number of the microscopic organisms are misidentified. Examining any culture collection's catalog will reveal a wide variety of samples which were accepted as one organism, yet found to be another. As a result, any new strain must be carefully examined to ensure the organism is correctly identified, regardless of the source. While this process is time-consuming and expensive, without the guarantee that a strain is correctly identified, the entire collection *raison d'être* is undermined. This fact is particularly true for a collection, such as the one proposed, which will be used as a 'standard' for comparison and identification of unknown pathogens.

### 4) Accessible

If a culture collection is to be useful, public users must be able to not only access the strains held in the collection, but also identify whether the collection holds any potentially useful strains. Therefore, collections need to provide the public with comprehensive documentation of the strains held and their characteristics. This information is best supplied in the form of a computer database, accessed using a web based search engine.

### III - Collection Holding Categories

Pathogens (and microscopic organisms in general) can be divided into a number of distinct and quite different groups. For the proposed pathogen oriented culture collection, four different groups of organisms exist: bacteria, viruses, fungi, and protozoa. Each group has its own characteristics and peculiarities, and can be addressed, in many senses, as distinct categories. For example, any collection holding bacteria will tend to operate in the same manner, require the same resources and funding, and employ specialists of a certain kind. Typically each group of organisms has significantly different manpower, skill and equipment requirements, with only limited overlap. This scenario is particularly true of technical skills and scientist training. As a consequence, organizing a single culture collection which contains both bacteria and viruses will likely have a cost similar to two collections, one holding bacteria, the other viruses. Thus, these four groups of pathogens form convenient 'biological divisions' which may match divisions within the overall collection organization.

The tables below summarize the four groups of organisms, and their principle features.

**Table IV Bacteria**

Requirements		Cost	
growth media (bacterial culture plates)		low	
culture conditions	aerobic	negligable	
	anaerobic	high	
identification	colony morphology		negligable
	microscopic morphology		low
	microscopic staining		low
	metabolism	requirements	low
		products	mid
	antigens		high
	nucleic acid	hybridization	mid
sequencing		mid	
contamination detection during growth		negligable	
storage	lyophilization (15 years+)	high/negligable	
	liquid nitrogen (unlimited?)	mid/low	

split costs indicate initial vs ongoing costs

Task	Difficulty
purification/eliminate contamination	low
identification of unknown organism	mid
confirmation of 'known' organism identity	low
growth of uncharacterized organism	mid
storage of uncharacterized organism	low

**Table 2 Viruses**

Requirements		Cost
growth media (tissue culture or others)		high
culture conditions (incubators)		mid
identification	plaque morphology	low
	TEM microscopic morphology	mid
	antibody staining	high
	antigens	high
	nucleic acid	hybridization
	sequencing	high
contamination detection during growth		high
storage	liquid nitrogen (unlimited?)	mid/low

split costs indicate initial vs ongoing costs

Task	Difficulty
purification/eliminate contamination	high
identification of unknown organism	high
confirmation of 'known' organism identity	mid
growth of uncharacterized organism	high
storage of uncharacterized organism	mid

**Table 3 Fungi**

Requirements		Cost	
growth media (bacterial culture plates)		low	
culture conditions		negligible	
identification	colony morphology	negligible	
	microscopic morphology	low	
	metabolism	requirements	low
		products	mid
	antigens	high	
	nucleic acid	hybridization	mid
sequencing		mid	
contamination detection during growth		negligible	
storage	lyophilization (15 years+)	high/negligible	
	liquid nitrogen (unlimited?)	mid/low	

split costs indicate initial vs ongoing costs

Task	Difficulty
purification/eliminate contamination	low
identification of unknown organism	mid
confirmation of 'known' organism identity	low
growth of uncharacterized organism	mid
storage of uncharacterized organism	mid



**Table 4 Protozoa**

Requirements		Cost
growth media (tissue culture or others)		low - high
culture conditions (incubators)		mid
identification	microscopic morphology	low
	microscopic staining	low
	antigens	high
	nucleic acid	hybridization sequencing
contamination detection during growth		high
storage	lyophilization (15 years+)	high/negligible
	liquid nitrogen (unlimited?)	mid/low

split costs indicate initial vs ongoing costs

Task	Difficulty
purification/eliminate contamination	high
identification of unknown organism	high
confirmation of 'known' organism identity	mid
growth of uncharacterized organism	mid
storage of uncharacterized organism	high

In general, bacteria and fungi are low-cost organisms to identify, culture, and store while both viruses and protozoa collections are far more expensive. In particular, viruses present unusual technical challenges during identification, and growth. Overall, bacteria are relatively easy to add to a collection and maintain in culture, while fungi offer a medium challenge. Viruses and protozoa are difficult and labour intensive organisms to add and maintain within a collection.

In Canada, adequate water purification and the absence of insect vectors render protozoa relatively unimportant as BW and health threats. As these organisms are difficult and expensive to culture, a governmentally administered and funded collection housing these organisms appears to be a low priority. As a consequence, no further analysis of the feasibility of a pathogenic protozoan collection was conducted.

## **IV - Project Scale / Expansion / Organization**

This report is intended to provide an outline for organizing a range of different possible culture collections. Specifically, this proposal examines two variables, collection size / scope, and collection access.

### **1) Collection Size / Scope**

Collection size / scope represents what subset of known organisms should be held by the collection. This variable can be represented as a range, from small to large. The size / scope of the collection greatly influences the range of persons who will benefit from the collection, as well the overall purpose of the collection.

#### **Small**

- A) BW pathogen collection - identified BW threats
- B) high threat pathogen collection - identified BW threats, level 3 and 4 containment human pathogens
- C) human pathogen collection - all bacteria, viruses and fungi known to cause disease in humans
- D) pathogen collection - all bacteria, viruses and fungi known to cause disease in any host organism
- E) general microbial collection - all known bacteria, viruses, and fungi

#### **Large**

### **2) Collection Access**

This variable indicates who is allowed to retrieve material from the collection. Again, this variable can be expressed as a range of options, from limited to open. Collection access significantly alters administrative costs and complications.

#### **Limited**

- A) government staff only - defence researchers, department of agriculture, and provincial and Federal government medical labs (including the NML)
- B) government staff and direct government employees / contractors - as above but including those non-government individuals directly employed in related government projects
- C) government staff and certified private individuals - as above, but including persons who complete a certification process to receive strains, (eg. academic researchers, private and industrial researchers, and private medical labs)
- D) unrestricted

#### **Open**

This report will not evaluate all the possible combinations of collection scope and collection access. In particular, a comprehensive general microbial collection is an extremely complex entity. Very few of such collections exist world-wide. As planning a human / BW pathogen oriented collection is the goal of this report, only collection size A, B, and C will be examined in detail. The possibility of collection expansion will be addressed at various points during the report. All levels of collection access will be discussed as appropriate.

Three combinations of collection size and collection access are particularly appropriate, and will be assessed in detail. These combinations are:

- 1) Canadian BioAgent / Biothreat Collection (CBBC) - a government only collection storing identified BW threats and high-risk medical pathogens.
- 2) Canadian Pathogen Collection (CPC) - a restricted access collection containing human pathogens.
- 3) Canadian Microorganism Collection Network (CMCN) - a network of culture collections including those containing viruses, bacteria, and fungi.

Each of these three combinations have advantages, drawbacks, and other issues which will be examined individually, along with brief suggestions concerning collection organization and operation. These three collection models will guide estimates of collection requirements and costs in succeeding sections of the report. Note that these three suggestions presume the collection will be associated with a larger 'parent' organization as discussed fully in the analysis of capital costs (Section VIII).

### **1) Canadian BioAgent / BioThreat Collection (CBBC)**

Scale - identified BW threats and high risk pathogens (Size B)

Access - government staff only (Access A)

Physical Location - National Microbiology Laboratory (NML), Defence Research and Development Canada Suffield (DRDCS) and / or agricultural pathogens at Agriculture Canada facilities.

Staff - minimal, as low as one scientist per organism type (bacteria, viruses, fungi), one technician, database administrator.

Administration - no dedicated administrators, committee of curators and other government researchers.

#### Advantages

-low cost for facilities and administration.

-due to the limited number of organisms held, comparatively easily set up.

#### Drawbacks

-limited collection size restricts value of strains for comparison purposes.

-ill suited to manage novel pathogen threats and recombinant / modified BW agents.

-may not have dedicated full-time staff, difficult to attract / maintain personnel.

-if dedicated scientists maintain collection, may not have sufficient work.

-may not use equipment / facilities to full capacity.

Two different CBBC collections have been evaluated, CBBC1, a 'type strain' collection which holds only one strain per pathogen, and CBBC2, a larger sized collection with a variety of strains per pathogen species. Realistically, any actual collection will likely fall somewhere between these two extremes, however these two models provide a useful bracket to evaluate staff, equipment, and costs. Functionally, the CBBC1 and CBBC2 models are very similar.

The CBBC approach is a low cost, minimum outlay collection intended for storing only the most serious pathogens with a correspondingly limited government user-pool. As this collection would include BL 4 containment organisms, it must be located, at least in part, at the NML. This kind of collection would have little general medical application, and represent more of a 'biological reserve' of otherwise unavailable pathogens, principally as research resources. The small number of holdings would greatly diminish the value of the collection for identification purposes. The small size of this collection would make staffing a challenge; at least one bacteriologist and one virologist would be required, yet the actual amount of biological material they would maintain would be quite small. While the least expensive option, the CBBC offers the lowest value in number of holdings per dollar, and would have the least value to the country as a whole due the collection neither being accessible nor comprehensive.

## **2) Canadian Pathogen Collection (CPC)**

Scale - human pathogens (Size C)

Access - government staff and employees (Access B) or also certified medical, academic and private researchers (Access C)

Physical Location - NML, DRDCS, and / or agricultural pathogens at Agriculture Canada facilities.

Staff - small, one or two scientists and several technicians per organism type (bacteria, viruses, fungi), database administrator.

Administration - committee of curators and other relevant government employers. If certified users allowed to access the collection, additional administration will likely be required.

Advantages

- collection size likely very useful for research and comparison purposes.
- efficient use of manpower, likely to attract/maintain trained personnel.
- efficient use of hardware and facilities.

Drawbacks

- collection size may drain resources/facilities from host facilities.
- if opened to the public, administration costs may be significant.
- likely will lack a mechanism to recover costs from external strain requests.

Two different CPC collections have been evaluated, CPC1, a 'type strain' collection in which holds only one strain per pathogen, and CPC2, a larger sized collection with a variety of strains per pathogen species. Realistically, any actual collection will likely fall somewhere between these two extremes, however these two

models provide a useful bracket to evaluate staff, equipment, and costs. Functionally, the CPC1 and CPC2 models are very similar.

The CPC contains a significantly larger number of organisms than the CBBC, and would be much more comprehensive and thus useful, especially for identification purposes. This collection could be conveniently split between installations, for example the viral component could be held at the NML while bacteria could be positioned at DRDCS. The need for the collection to be physically unified is less important than other considerations such as staff, space, facilities and resources. Staff and equipment are employed efficiently considering the overall number of organisms held. A special issue when planning the CPC is whether the collection should include a fungal component. Canada already has a fairly extensive medical fungal collection, the University of Alberta Microfungus Collection (UAMC). Options involving the UAMC will be discussed throughout the report.

### **3) Canadian Microorganism Collection Network (CMCN)**

As the CMCN approach has two different levels of organization, it represents a very different strategy from the CBBC and CPC. The CMCN contains a central administrative and coordination organization (the CMCN itself) which serves a number of individual culture collections scattered across the country. The network is intended to include government and non-government (academic and private) collections. While this dispersed collection may initially seem inefficient, this strategy of a central umbrella organization supporting collection nodes has proven extremely successful in a number of countries including Belgium (Belgian Co-ordinated Collections of Micro-organisms (BCCM)) and the United Kingdom (United Kingdom National Culture Collection (UKNCC)). The alternative, a large central national culture collection was favoured during early collection development, the classic example being the American Type Culture Collection. (ATCC). Advances in communications have made a more decentralized approach possible, if not preferable. The CMCN strategy seeks to have the maximum range of holdings while retaining a core pathogen focus. Similarly, access would be as wide as possible.

The proposed CMCN would initially be composed of the following elements:

- A) Canadian Microorganism Collection Network - the network hub / administration
- B) Canadian Pathogen Collection - the same collection as described above, only now part of a larger network
- C) Additional government collections
- D) Private collections

Each of these components will be examined in detail, followed by an analysis of the overall value of the CMCN strategy.

## A) Canadian Microorganism Collection Network

Scale - no organisms held by this coordinating agency

Access - inapplicable

Physical Location - may be located anywhere, but preferably with the CPC for coordination purposes

Staff - director / administrator (possibly a CPC curator), small (1-3) office / clerical staff, 1 database administrator.

Administration : director / administrator, assisted by committee of member collection curators and other government employees.

Functions :

- 1) coordinate culture collections, in particular funding, information management and access.
- 2) maintain a central standardized database for collections, and provide the public with search tools for the database.
- 3) provide an interface for those requesting strains and certify access to particular samples and collections (eg. pathogens).
- 4) set standards for accredited culture collections (issues such as quality control, storage standards, restrictions on dispatch of samples).
- 5) provide a mechanism for new collections to be identified, funded, and added to the network.
- 6) create protocols and standards for security issues.
- 7) operate a cost recovery and billing system for both private and government collections.
- 8) assist in hiring replacement staff (retirements / transfers) and possible transfers of 'endangered' collections to other nodes in the network.
- 9) assess the 'usefulness' of collections (scientific, technological, medical, etc)
- 10) lobby for funding then distributed throughout the entire network.
- 11) develop government regulatory policies and standards which assist culture collections as a whole, (eg. standards for postal services and customs handling of culture collection materials).
- 12) provide long-term planning and development for culture collection and genetic resources.
- 13) act as Canada's representative in international culture collection organizations and committees.

The CMCN would provide the following centralized services to subordinate culture collections

- 1) provide information access, maintain and operate a central information database.
- 2) process external requests for strains.
- 3) recover costs from external requests.
- 4) coordinate with other collections.
- 5) act as a pipeline for funding from the Federal government.
- 6) act as an interface with other governmental agencies.

### B) Canadian Pathogen Collection (CPC)

As described see option 2, above.

### C) Additional Government Culture Collections

These collections are principally those administered by Agriculture Canada but may also include provincial holdings (both agriculture and medical). In particular the Canadian Collection of Fungal Cultures houses organisms which could serve as agriculture BW agents. Provincial medical lab collections may also be incorporated in this manner. Aside from the services provided by the CMCN, these collections would remain independently staffed, administered and maintained.

### D) Private (academic and industrial) Collections

These collections could include a diverse set of holdings identified by the CMCN as suitable to participate within the network. The UAMC is ideally suited to be the initial participant given its medical fungal collection. Again, aside from services provided by the CMCN, these collections would remain independently staffed, administered, and maintained.

### Advantages

- provides government agencies access to collections and expertise without committing dedicated funds, facilities, staff.
- allows efficient centralization of resources and processes (eg. cost recovery, database, security issues).
- allows for flexible expansion.
- provides a superior funding mechanism for Canadian culture collections.
- creates standards for culture collections, allows for long-term planning.
- collections within the network are more accessible, and stable.

### Drawbacks

- most expensive option

The CMCN strategy is more than a simple pathogen collection, but instead an attempt to address many long-standing issues faced by Canadian culture collections. Any smaller pathogen collection model will inevitably face the handicaps encountered by existing Canadian culture collections. In that sense, the CMCN is a practical structure ensuring any newly created pathogen collection is able to operate as efficiently as possible.

A network of culture collections assisted by an umbrella organization offers more than an efficient mechanism for providing access to pathogen specimens. This strategy offers the possibility of creating continuity and stability for a set of resources which has experienced only the opposite. Worldwide, culture collections are being lost due to financial limitations or when a researcher or an institution loses interest in maintaining a collection. This crisis is all too well recognized within the culture collection community, for example, the World Federation for Culture Collections (WFCC) maintains a 'culture in distress' assistance group (19). Unfortunately, this assistance is typically limited to logistical and lobbying advice, and guidance on emergency damage control.

Scientifically, the results are both tragic and catastrophic. Resources generated over literally decades of research are destroyed or allowed to decay. Canada, sadly, is no exception. A detailed collection survey conducted in 1986 (20) identified 136 collections within Canada, 23 of which were considered 'strategic', containing unique holdings of international significance. In 1994, only 86 remained. Today, the number is even lower. A sadly typical example is the fate of the tree pathogen fungal collections held by National Resources Canada. These have not been examined or recultured for years due to manpower and funding cutbacks. Typically only a few thousand dollars are required to save a culture collection, but those funds are often not available. Most collections depend on periodic (typically every one to three years) grants from government (NSERC) or their host institutions. Unfortunately, these grants fall in an awkward size, too large to be a casual expense, yet too small to qualify for strategic or infrastructure programs. As a consequence, culture collections in Canada usually operate in a hand to mouth manner, each year barely meeting needs and unable to engage in long-term planning. This fact, more than any other, was the central complaint raised at the June 24, 2003 Status of Microbial Genetic Resources and Culture Collections in Canada Workshop held in Montreal (21, 22, 41).

By presenting the total fiscal requirements and other needs of the Canadian culture collection community, the CMCN can provide a more effective method of obtaining government action. Importantly, funding should become more stable, providing at a minimum 10 year grants / plans. Without this foundation, any national culture collection program is simply ill advised.

The CMCN can also reduce collection costs and simultaneously increase collection utility by providing information, administration, and database services. Data management has long been recognized as a service required by Canadian culture collections. The 1989 Task Force on the Status of Culture Collections in Canada report to the Minister of State (Science and Technology) (23) recommended creating a national culture collection holding database. 61% of all culture collection curators identified database support as a crucial need, second only to a need for improved funding (70% of respondents.) This complaint remains a significant issue (21). Culture collections are often relegated to using out of date and / or poorly supported software. With Internet technology, a well-managed and technologically up-to-date database may be designed and then shared by many collections, not only offering a single online source for those accessing the collection, but also providing an essential and low-cost tool required by all Canadian culture collections. The CMCN is the ideal agency to create and manage such a database.

The CMCN is also an efficient cost-recovery tool. Federal government collections are not allowed to collect payment for samples issued, but the CMCN could provide this service. Administering the process of making purchases, especially online, is itself costly and time-consuming for individual collections. By centralizing strain purchases through the CMCN, collections merely are responsible for actually shipping samples. Access to collections, especially those with pathogens, is another significant complication given the recent evolution of terrorist threats. Culture collection curators, both private and public, can hardly be expected to determine whether recipients are legitimate and / or whether a lab is certified. The CMCN could take on this task, setting standards for certifying access to pathogens, and determining whether a particular request



should be granted. This activity should be coordinated with the appropriate health and security administrations of the Federal and provincial governments.

The CMCN also can provide a voice for the collections as a group. Culture collections have particular needs and requirements, especially in regards to government regulations. The CMCN can represent collection needs on long-standing problems (24) such as export / import procedures and packaging and safety issues for samples in the postal system. On the international stage, the CMCN can act as a representative of Canada's culture collections.

The initial proposed CMCN structure concentrates on collections providing pathogens: the new CPC for viruses and bacteria, Agriculture Canada's fungal collections, and the UAMC. This set of collections provides a useful and complete unit for medical and BW needs. However, the CMCN organization can be expanded further to incorporate additional Canadian culture collections, including those with an industrial or scientific focus. This growth would be a benefit to all parties; collections joining the CMCN would obtain funding, as well as information and administrative support, while the collection holdings become more accessible to the public and crucially, more stable. Ideally, the CMCN could support and protect both collections which have a 'strategic' value, and those which are essential requirements for society and the state. Thus long term planning, growth and development of genetic / biological resources within Canada could occur.

## V - Pathogen Lists and Analysis

Assessing project requirements first requires making an estimate of the living resources a proposed collection will hold. This estimate is vital, as the type and number of organisms held determines most of the resulting costs and infrastructure. As the collections proposed in this report focus on pathogenic microorganisms, an assessment of the approximate number of human pathogens was necessary. Four pathogen lists were constructed:

- 1) high risk pathogens / BW agents
- 2) human bacterial pathogens
- 3) human viral pathogens
- 4) human fungal pathogens

In each case, a list of all organisms within the category was compiled, along with additional facts which might prove valuable during collection planning.

Any attempt to generate an 'accurate' or 'canonical' list was impossible as taxonomic studies reassign, split and merge species and new pathogens emerge. Indeed, even defining a 'pathogen' is difficult. Should pathogens include organisms known to infect humans asymptotically and / or opportunistic infections seen in immunocompromised individuals? For this study, a very liberal definition of pathogen was used to provide figures which err on the side of caution, rather than underestimate collection size and needs. As a consequence, all organisms conventionally identified as pathogens were counted. Pathogenic viruses include any agent which causes fever and / or more acute symptoms. The initial source for pathogen species data was Zinsser Microbiology (24). For viruses, Field's Virology (25) and Mahy's Dictionary of Viruses (26) were important sources of supplementary data. Lists were then modified and amended following many other literature sources, particularly those addressing species specific issues. In all cases, extensive revision of taxonomy and nomenclature occurred while the lists were being assembled. Whenever possible up-to-date taxonomic information was used. Nevertheless, caution is advised when referring to these lists and the names listed there-in. Many culture collections do not maintain their catalog listings with 'current' names, and in many cases organisms are filed by older synonyms. Consequentially, these lists are approximations intended for feasibility planning purposes only, though this data may ultimately also prove useful for prospective curators when establishing collections.

The agent lists allow calculation of the number of pathogenic organisms within each groups, specifically addressing three different values:

- 1) the number of different pathogenic species
- 2) the number of different strains of pathogenic species
- 3) the number of different strains within the pathogen's genus

The number of pathogenic species could be determined simply by examining the appropriate literature. The number of different strains was estimated by surveying public

culture collection catalogs and counting the different isolates of a given species. The number of different strains within a given pathogen's genus was determined in an analogous manner, for example, the total number of *Bacillus* strains in a collection was determined for *Bacillus anthracis*. Note, some pathogenic species, such as *E. coli*, have been the targets of extensive research and modification, generating a large number of strains, sometimes in the thousands. These figures do not indicate a wide variety of distinct pathogenic strains. In these cases, strain counts were arbitrarily reduced to 100, though the original data was retained for reference purpose. Some pathogen species which clearly exist within culture collections had been 'hidden', likely as a response to current bio-terrorism concerns. These species were counted as their own distinct category.

The 'strains per pathogen' and the 'strains per pathogen genus' values are by necessity estimates only. However, surveying a number of large culture collections allowed an approximate idea of what would be housed in a typical well-stocked collection. A list of the culture collections used during the survey is presented in Appendix XI, along with collection details.

Two crucial values were derived during these calculations, the number of strains, and the cost / complexity adjusted number of strains. Again, these values were not intended to be absolutely accurate figures. The number of strains is simply the number of different organisms or isolates which exist within a category. The cost / complexity adjusted number of strains value reflects the fact not all strains or organisms are equally easy to maintain in a culture collection. For example, a bacterium which requires an unusual growth substrate or medium is assumed to cost twice as much to maintain as a 'normal' bacterium. These additional costs and complications act as 'multipliers', and increase the effective requirements of a particular specimen. For example, a collection may hold 30 different bacteria, but 5 only grow in anaerobic conditions and another 3 may require unusual growth media. Therefore, the cost / complexity adjusted strain number would be 38 strains. These cost / complexity multipliers are approximations at best, they are not intended to be extremely precise reflections of the cost of maintaining a strain.

Thus, the number of strains a collection holds is a calculation of the absolute number of organisms within a collection. The cost / complexity adjusted strain number indicates roughly what resources are required to maintain those strains. Both values are used throughout the report when appropriate.

The cost / complexity multipliers are different for each organism group, and were identified by literature investigation. The individual multipliers are listed and discussed below.

### Bacteria

- A) Complex / Unusual Needs - species requires unconventional growth media and / or nutrients. "Standard" growth media include common bacterial media (eg. trypticase soy agar, and standard medical microbiology media (eg. blood agar, chocolate agar.)
- B) Unusual Atmosphere / Anaerobic Species - species requires an altered atmosphere for growth. In bacteria, these organisms are typically microaerophiles or

anaerobes and thus must be maintained in a low oxygen or oxygen free environment.

- C) Parasitic Species - species only grows when parasitically associated with another organism, typically living inside the host cell. For human pathogens, parasitic species are usually grown in tissue culture in a manner analogous to viruses.
- D) BL 3 Species - species requires BL 3 containment procedures for basic handling and / or growth in bulk.

### Fungi

- A) Complex / Unusual Needs - species requires unconventional growth media and / or nutrients. "Standard" growth media are common fungal agars such as Sabourand's agar and sheep blood agar.
- B) Unusual Atmosphere / Anaerobic Species - species requires an altered atmosphere for growth. All fungi are aerobic, however some sporulate better in a modified atmosphere.
- C) Parasitic Species - species only grows when parasitically associated with another organism.
- D) BL 3 Species - species requires BL 3 containment procedures for basic handling and / or growth in bulk.

### Viruses

- A) BL 3 Species - species requires BL 3 containment procedures for basic handling and / or growth in bulk.
- B) BL 4 Species - species requires BL 4 containment procedures for basic handling and / or growth in bulk. The BL 4 containment multiplier is a 'double' multiplier to reflect the extreme costs and difficulty involved in managing this very dangerous category of organisms.

As all viruses are parasitic, and require complex growth procedures (tissue culture, egg inoculation, etc) the first three multipliers were not used when calculating virus cost / complexity strain numbers.

## **Pathogen Lists / Strain Numbers**

### 1) High Risk Pathogens / BW Agents

This list of organisms includes pathogens which either represent very unusual medical risks to both the public and medical profession, or have been identified as likely BW agents. High risk organisms were identified by their assigned containment levels (BL 3 and BL 4). No "official" Canadian BW agent list has been published in open literature. Consequentially BW agents were identified by merging two published lists; NATO Handbook on Medical Aspects of NBC Defensive Operations (Appendix A) (27) and the Center for Disease Control (CDC) (28). A modified version of these lists is presented in Appendix I. The complete high risk pathogen / BW agent list and associated

data is found in Appendix VII. The number of different species is summarized in Table 5 below:

**Table 5 Summary of BL3/4 and BW Species Data**

<b>Bacteria</b>	
Species	38
Unculturable Species	0
Culturable Species	<b>38</b>
Complex / Unusual Needs Species	12
Unusual Atmosphere / Anaerobic Species	5
Parasitic Species	13
BL 3 Species	30
Number Cost / Complexity Adjusted Species	<b>98</b>

<b>Fungi</b>	
Species	3
Unculturable Species	0
Culturable Species	<b>3</b>
Complex / Unusual Needs Species	0
Unusual Atmosphere / Anaerobic Species	0
Parasitic Species	0
BL 3 Species	3
Number Cost / Complexity Adjusted Species	<b>6</b>

<b>Viruses</b>	
Species	61
Unculturable Species	0
Culturable Species	<b>61</b>
BL 3 Species	40
BL 4 Species	17
Number Cost / Complexity Adjusted Species	<b>135</b>

These values were used to estimate the size of the Canadian BioAgent / BioThreat Collection type strain collection (CBBC1), a collection with only one representative strain per pathogen species. The size of the expanded CBBC2 collection was determined by identifying the number of different strains of each pathogen present in a variety of world culture collections, see Appendix VII for individual species. The results are summarized in Table 6 below :

**Table 6 Summary of BL3/4 and BW Strain Data**

<b>Bacteria</b>			
Culture Collection Examined	ATCC	UKNCC NCTC	BCCM
Different Pathogenic Species Strains	611	592	85
Number Cost / Complexity Adjusted Species Strains	1277	1128	141

Estimated Strains in Collection	<b>500</b>
Estimated Strains in Collection Adjusted for Cost / Complexity	<b>1000</b>

<b>Fungi</b>				
Culture Collection Examined	ATCC	UKNCC NCTC	CBS	UAMH
Different Pathogenic Species Strains	133	114	135	13
Number Cost / Complexity Adjusted Species Strains	195	151	170	16

Estimated Strains in Collection	<b>100</b>
Estimated Strains in Collection Adjusted for Cost / Complexity	<b>200</b>

<b>Viruses</b>		
Culture Collection Examined	ATCC	UKNCC NCPV
Different Pathogenic Species Strains	70	95
Number Cost / Complexity Adjusted Species Strains	117	156

Estimated Strains in Collection	<b>100</b>
Estimated Strains in Collection Adjusted for Cost / Complexity	<b>150</b>

## 2) Human Bacterial Pathogens

This pathogen list includes all bacteria species known to infect humans and cause disease. The complete bacterial pathogen list is found in Appendix VIII. Bacterial species data is summarized in Table 7.

**Table 7 Summary of Pathogenic Bacteria Species Data**

Species	226
Unculturable Species	4
Culturable Species	<b>222</b>
Complex / Unusual Needs Species	45
Unusual Atmosphere / Anaerobic Species	52
Parasitic Species	17
BL 3 Species	30
Number Cost / Complexity Adjusted Strains	<b>366</b>

These values were used to estimate the bacterial component of the Canadian Pathogen Collection type strain collection (CPC1). Table 8 (below) shows the results of the world culture collection pathogenic bacteria strain comparison. Estimates for the CPC2 bacterial holdings and a broader 'general' collection are indicated. The CPC2 value represents the total number of different strains of pathogenic bacteria identified, while the general collection value is the number of strains which belong to the same genus as a known pathogenic bacterium.

**Table 8 Pathogenic Bacteria Collection Survey**

Culture Collection Examined	UKNCC		
	ATCC	NCTC	BCCM
Pathogen Species Missing from Collection	10	41	91
Pathogen Species Restricted / Hidden	25	22	27
Pathogen Species Present	191	161	107
% Pathogen Species in Collection	85%	71%	48%
Pathogenic Species Strains	3231	2531	1655
Number Cost / Complexity Adjusted Species Strains	4697	3532	1895
Pathogenic Genus Strains	11618	9081	8000
Number Cost / Complexity Adjusted Genus Strains	16386	11607	9486
	Strains	Cost/Complexity Adjusted Number	
Estimated Strains in CPC2 Collection	<b>2500</b>	<b>3000</b>	
Estimated Strains in General Collection	<b>10000</b>	<b>12000</b>	

### 3) Human Viral Pathogens

This list includes all viruses known to infect humans and cause disease. The complete pathogenic virus list is found in Appendix IX. Species data is summarized in Table 9.

**Table 9 Summary of Pathogenic Virus Species Data**

Species	160
Unculturable Species	0
Culturable Species	<b>160</b>
BL 3 Species	41
BL 4 Species	16
Number Cost / Complexity Adjusted Strains	<b>232</b>

These values define the viral component of the Canadian Pathogen Collection type strain collection (CPC1). Table 10 (below) shows the results of the world culture collection pathogenic virus strain comparison. Estimates for virus CPC2 and a broader 'general' collection are indicated. The CPC2 value represents the total number of different strains of pathogenic viruses identified, while the general collection value is the number of strains which belong to the same genus as a known pathogenic virus. Note that this survey only included two virus collections; worldwide animal and human virus collections are extremely rare due to their complexity and cost. Only these two collections offered searchable catalogs which made a holding survey feasible.

**Table 10 Pathogenic Virus Collection Survey**

Culture Collection Examined	ATCC	UKNCC NPVC
	Pathogen Species Missing from Collection	72
Pathogen Species Present	87	51
% Pathogen Species in Collection	45%	33%
Pathogenic Species Strains	455	406
Number Cost / Complexity Adjusted Species Strains	512	473
Pathogenic Genus Strains	910	412
Number Cost / Complexity Adjusted Genus Strains	1031	483
	Strains	Cost/Complexity Adjusted Number
Estimated Strains in CPC2 Collection	<b>450</b>	<b>500</b>
Estimated Strains in General Collection	<b>750</b>	<b>750</b>

**3) Human Fungal Pathogens**

This list includes all fungi known to infect humans and cause disease. The complete list of pathogenic fungi is found in Appendix X. Species data is summarized in Table 11.



**Table 11 Summary of Pathogenic Fungi Species Data**

Species	104
Unculturable Species	2
<b>Culturable Species</b>	<b>102</b>
Complex / Unusual Needs Species	0
Unusual Atmosphere / Anaerobic Species	0
Parasitic Species	0
BL 3 Species	1
<b>Number Cost / Complexity Adjusted Strains</b>	<b>103</b>

These values define the fungal component of the Canadian Pathogen Collection type strain collection (CPC1). Table 12 (below) shows the results of the world culture collection pathogenic fungus strain comparison. Estimates for the CPC2 fungal holdings and a broader 'general' collection are indicated. The CPC2 value represents the total number of different strains of pathogenic fungi identified, while the general collection value is the number of strains which belong to the same genus as a known pathogenic fungus. The UAMH column represents the human pathogen holdings of the University of Alberta Microfungus Collection, the collection proposed as part of the CMCN network.

**Table 12 Pathogenic Fungi Collection Survey**

Culture Collection Examined	ATCC	UKNCC NCTC	CBS	UAMH
Pathogen Species Missing from Collection	5	14	32	37
Pathogen Species Restricted / Hidden	0	0	0	0
Pathogen Species Present	98	89	71	66
% Pathogen Species in Collection	95%	86%	69%	64%
Pathogenic Species Strains	2485	2013	1774	1089
Number Cost / Complexity Adjusted Species Strains	2485	2013	1786	1098
Pathogenic Genus Strains	12548	5273	3677	2377
Number Cost / Complexity Adjusted Genus Strains	12548	5273	3689	2386
		Strains	Cost/Complexity Adjusted Number	
Estimated Strains in CPC2 Collection		<b>2000</b>	<b>2000</b>	
Estimated Strains in General Collection		<b>5000</b>	<b>5000</b>	

## VI - Culture Procedures

Worldwide, culture collections have developed efficient long-standing procedures to ensure key collection are comprehensive, stable, accurate, and accessible. These procedures are essentially the same for all organism groups (bacteria, viruses, fungi) as they represent the methods by which a collection ensures the quality and accuracy of their holdings. Three primary culture collection procedures exist:

- 1) new strain acquisition
- 2) maintenance of collection strains (testing viability, re-culturing)
- 3) distribution of collection samples

For this report, these protocols are important as they affect hardware and manpower requirements, as well as represent key procedures of any formal culture collection.

Standard collection procedures are well developed in culture collection literature. A number of texts which proved particularly useful in developing the 'theory' and minimum requirements for culture collection procedures are listed in Appendix II. In particular, Hill and Kirsop, *Living Resources for Biotechnology: Bacteria* (29), is highly recommended. The remainder of the Living Resources series may also be helpful. The database procedure outline in Appendix IV also traces these procedures, and is useful as a reference to fill out the individual steps involved in routine culture operations.

### 1) Stock Acquisition

Any culture collection can be expected to add new samples, both during the initial development of the culture, and as the collection expands. The complexity of adding a new strain will likely vary considerably depending on the source of the strain. Those organisms which are well characterized and have established growth / culture characteristics, such as those obtained from other culture collections, will likely require little effort. Newly identified strains may require research to determine appropriate growth and preservation techniques, as well as confirm the organism's identity. The following checklist is suggested for new strain acquisitions:

- 1) Enter preliminary strain information into the database, assign the specimen master collection number, taxonomic information, strain source and / or donor.
- 2) Grow the strain.
- 3) Confirm:
  - A) the organism is viable
  - B) the organism is not contaminated. If contaminated, the culture is re-grown under selective conditions. This can be achieved by plating / streaking on non-selective solid media for bacteria and fungi. Virus samples may be purified by plaque assay.
  - C) the organism is correctly identified. See below for an extended discussion of organism identification techniques.

- 4) Enter the full specimen information within the database. This information should include:
  - A) organism name, alternative organism names, obsolete organism names, basic taxonomic description.
  - B) organism growth and culture characteristics, requirements.
  - C) photo of colony morphology, and written description.
  - D) photo of organism, and written description.
  - E) other unusual characteristics/features.
  - F) if nucleic acid information obtained in step 3C, add to the database. In the case of small genomes (viruses) this may extend to complete genomes, though for bacteria and fungi data will likely be restricted to rRNA sequences.
- 5) Grow the specimen in a large scale for storage. In most cases the growth conditions will have been determined previously. If not, some experimentation may be required. Literature sources in Appendix II are suggested. The number of viable cells / viruses per ml. should be established.
- 6) Storage. The vast majority of specimens will likely be either frozen (in freezers or liquid nitrogen) or lyophilized (freeze dried) or both. See below for an extended discussion of organism storage techniques.
- 7) Viability Confirmation: once samples of the strain have undergone storage, one aliquot should be revived and the number of remaining viable cells / viruses determined. This step ensures that the storage process has not resulted in an unacceptable decrease in the number of viable cells. Should too few living organisms remain, steps 5, 6 and 7 should be repeated.
- 8) Add detailed storage labels (possibly with barcodes) Complete database information for the strain, enter information for storage locations (freezer, box, vial number, etc).

Two significant issues exist within the stock acquisition process: confirming strain identity and how to preserve and store samples.

### Strain Verification

Strain verification and the related issue of contamination detection / identification is a major concern for culture collections. Classically, organisms are identified by characteristics such as morphology (microscopic and colony), growth characteristics, metabolic and biochemical properties, mobility, resistance, antigenic features, and a wide variety of other organism characteristics. Contamination is typically assessed by growth on non-selective solid media, then identifying which colonies / plaques represent the desired specimen. Organism morphology is determined microscopically; phase contrast microscopy is particularly useful for assessing bacterial flagella, while transmission electron microscopy is required for determining virus morphology. Antibody binding can be used to either detect and quantitate organisms, or allow direct visualization by immunofluorescent microscopy. These techniques are time consuming, expensive, and require a wide variety of reagents, media, and other supplies. However, these traditional approaches will likely prove necessary in some situations.

The development of molecular biology techniques has offered an alternative, identification through the direct sequencing of nucleic acids. Sequencing the rRNA

genes of the target organism is the most common method. This process involves first isolating organism DNA, amplifying with a standard set of PCR primers, and sequencing the product. As rRNA sequences are the most common target of genomic studies, an unknown sample's rRNA sequence allows comparison with a very large library of known and characterized species. Some quantification of sample concentration is also possible using real-time PCR techniques. This rRNA analysis is particularly well suited for identifying bacteria, and fungi; commercial kits and software have been developed to help automate the process (eg. Applied Biosystem's MicroSeq® Microbial Identification System) (30). Incorporating rRNA sequencing as an identification method is strongly recommended for a number of reasons:

- A) Universality - unlike the classic identification techniques which may require unusual species or strain specific media and supplies, rRNA sequencing uses the same reaction components, apparatus and supplies for all bacteria, and fungi. Cost during identification is therefore reduced.
- B) Stability - many of the characteristics used in classic identification are prone to change, especially in cultures maintained in un-natural laboratory conditions. This factor significantly adds to confusion and uncertainty when determining strain identity. rRNA sequences are very stable and unlikely to undergo significant change.
- C) Taxonomic Standards - rRNA and other nucleic acid and protein sequences are increasingly the standard method by which phylogeny is assessed scientifically. Determining rRNA sequences of samples held within the collection therefore increases the scientific value and utility of the collection for taxonomic as well as identification purposes.
- D) Real-time PCR of mRNA sequences allows analysis of characterized metabolic phenotypes without resorting to expensive biochemical tests (31).
- E) Speed - nucleic acid analysis is extremely fast (current techniques can produce data in under 24 hours) and does not require the strain be successfully grown. A newly received sample can be sequenced at any time during the acquisition process.
- F) Future Technological Progress - given the explosive development of molecular biology, in particular nucleic acid techniques, the ease of obtaining nucleic acid information can only be expected to increase, along with a corresponding decrease in costs. A nucleic-acid intensive approach to collection maintenance offers the opportunity to adopt a powerful new technology with tangible current benefit and still more future promise.

Applying current nucleic acid identification techniques to viral samples is more problematic. Viruses lack any common required conserved nucleic acid sequences analogous to the rRNA of fungal and bacteria. Therefore, each virus may require a

unique set of PCR identification and sequencing primers. This additional cost may not prove exorbitant given the limited number (160) of human pathogenic virus species. As well, classic methods of virus identification (morphology, antigenic characteristics) are both imprecise and expensive in comparison to classical fungal and bacterial identification techniques. Thus, nucleic acid identification will likely prove advantageous for virus isolates as well.

DNA molecular biology techniques are also useful in detecting less obvious forms of contamination, such as mycoplasma in viral samples. As these bacteria are parasitic, merely plating a sample on non-selective media will not detect mycoplasma. However, PCR can easily detect the presence of characteristic mycoplasma DNA sequences.

Future technological advances may offer additional alternatives. As virus genomes are typically small (several thousand base-pairs), determining complete genome sequences may not prove out of the question during the strain acquisition process. Collection curators are strongly advised to consider the implications of technological developments in respect to improving culture identification.

Preservation / Storage Methods

A wide variety of microorganism preservation techniques have been developed over the last century, each with particular advantages and drawbacks. These techniques are extensively reviewed in the texts listed in appendix two. Bacterial preservation techniques are summarized in Table 12, fungal procedures in Table 13.

**Table 12 Methods of Preserving Bacteria**

Method of Preservation	Cost of Material	Labour	Longevity	Genetic Stability
Serial transfer on agar				
Storage at room temp	low	low	1-4 weeks	poor
Storage in refrigerator	mid	low	1-6 months	poor
Drying	mid	mid	4-12 months	fair
Freezing	mid	low	highly variable sometimes years	fair
Lyophilization	high	initially high, then negligible	3-80 years	good to fair
Cryopreservation	high	low	infinite?	excellent

(various sources)

**Table 13 Methods of Preserving Fungi**

Method of Preservation	Cost Material	Labour	Longevity	Genetic Stability
Serial transfer on agar				
Storage at room temp	low	low	1-6 months	poor
Storage in refrigerator	mid	low	6-12 months	poor
Storage under oil	low	low/mid	1-40 years	poor
Storage in water	low	low/mid	2-5 years	fair
Storage in deep freeze	mid	low/mid	4-5 years	fair
Drying				
In soil	low	mid	5-25 years	poor to fair
Silica Gel	low	mid	5-19 years	good
Lyophilization	high	initially high, then negligible	4-40 years	good to fair
Cryopreservation	high	low	infinite?	excellent

(source : Smith D., and Kolkowski, J. Fungi, in *Maintaining Cultures for Biotechnology and Industry*, eds. Hunter-Cevera, J.C. and A. Belt, 1996, Academic Press, NY)

Given the culture collection's need to maximize strain viability and stability, lyophilization and liquid nitrogen cryogenic preservation are the preferred procedures. Storage in liquid nitrogen is particularly ideal, indeed, the upper limit of viability is not clear. Many samples stored over 50 years ago retain high viability. Cryopreserved samples also retain their original genetic and phenotypic characteristics. Lyophilized samples have lower but still impressive lifetimes, and require lower ongoing storage costs, typically being stored in refrigerators. Some research, however, has indicated that for at least some bacteria, the lyophilization procedure induces mutations, and may also select subpopulations which are particularly resistant to freeze-drying (32), making this technique significantly less attractive should strain stability be a primary issue, as for a culture collection.

In summary, for many fungal and bacterial species, either cryopreservation or lyophilization may be acceptable options, however liquid nitrogen cryopreservation should be used whenever costs are manageable. Many culture collections improve redundancy and stability by storing samples with two or more preservation methods. Ideally, the planned collection should adopt this strategy and use both lyophilization and cryopreservation.

Preservation options for viruses are more limited, typically cryopreservation, but occasionally freeze-drying or a combination of cryopreservation and freeze-drying (23). Table 14 lists the specific conditions developed for a small variety of viruses. Note the wide variation in stabilizing agents.

**Table 14 Virus Preservation Conditions**

Virus	Stabilizer	Storage Conditions
Herpes	serum, 7% DMSO in culture medium	-70 frozen
Herpes	sodium glutamate	-20 freeze-dried
Varicella-zoster	sucrose, sodium glutamate, albumin, KPO4	-70 frozen
Dengue	20-50% fetal bovine serum, 0.75-2.0% bovine albumin	-70 frozen
Sindbis	20-50% fetal bovine serum, 0.75-2.0% bovine albumin	-70 frozen
Measles	serum, DMSO	-70 frozen
Vesicular Stomatitis	serum, DMSO	-70 frozen
Respiratory syncytial	MgSO4 and HEPES buffer	-70 frozen
Adenovirus	culture medium	-70 frozen
Poliovirus	culture medium	-70 frozen
Poliovirus	dialysis low salts	-20 freeze-dried
Vaccinia	culture medium	-70 frozen
Influenza	serum	-70 frozen
Influenza	0.5% gelatin	-4 to -20 freeze-dried
Parainfluenza	0.5% bovine albumin, serum	-60 or less, frozen
Mumps	chicken amniotic fluid	-70 frozen
Rabies	0.75% bovine albumin, serum	-70 frozen
Hepatitis A	2-40% stool suspension	-70 frozen
Hepatitis A	culture medium	-70 frozen

(source :Beeler, J. A. Human and Animal Viruses, in Maintaining Cultures for Biotechnology and Industry, eds. Hunter-Cevera, J.C. and A. Belt, 1996, Academic Press, NY)

Preserving a previously uncharacterized virus often involves a good deal of trial and error. Literature data indicates that viruses are well preserved by storage in liquid nitrogen or  $-70^{\circ}$  C freezers, with no apparent upper time limit. Given the high costs of virus production, virus storage ideally should preserve large numbers of vials / ampoules preserved via cryopreservation.

In conclusion, cryopreservation is the optimal standard method of preservation. Samples can be stored either directly in liquid nitrogen or in the cold nitrogen vapour layer above. The latter method is preferable, as should liquid nitrogen leak into vials, samples can be damaged, and vials can explode once the vial is warmed, though for very valuable samples, direct immersion in liquid nitrogen may still be used. Alternatively,  $-80^{\circ}$  to  $-60^{\circ}$  C freezers may be used to supplement liquid nitrogen storage. These freezers have intrinsic risks as power outages can result in rapid and significant temperature rises. In comparison, liquid nitrogen storage offers, at minimum, several days of stable temperature should service interruptions occur.

## **2) Viability Checks / Re-culturing Strains**

For a collection to be useful, the stored organisms must remain alive. The number of living cells / viruses must be confirmed periodically to track loss of strain viability. This method essentially duplicates strain viability tests discussed during the stock acquisition process. A vial or ampoule is retrieved from storage, and the number of viable organisms remaining is quantified by either colony (bacterial/fungal) or plaque (virus) count. Data obtained is entered in the database. Should the number of organisms remaining alive be adequate, the stock remains in storage. If not, the organism is retrieved from storage, and re-grown (see below.)

Fortunately, viability checks need not be frequent. The first viability check should likely be one year following storage. If the strain appears stable, later viability checks need not be as frequent. Tracking the decrease in viable organisms can be used to determine whether a culture should be re-grown. The long-term viability of cultures obtained from other collections will often already be well characterized.

Once a strain's viability has dropped below a key threshold, the organism must be retrieved from storage, and re-grown. This process is essentially identical to steps 5, 6 and 7 of the stock acquisition process; growing the organism, counting the number of organisms/ml, storing the organism, and then determining the remaining viable organisms/ml. New information on strain holdings is entered into the database. Optionally, this process may include quality control tests as well; re-confirming the organism is uncontaminated and correctly identified. Re-culturing may prove a useful opportunity to apply new quality control procedures to older holdings, as new techniques are adopted.

## **3) Distribution of Collection Samples**

Once a strain is requested, a number of steps are necessary prior to distribution. The potential recipient must be qualified to receive the isolate, a crucial factor in the case of dangerous pathogens. Certification should not be an issue when the recipient is a government agency. The issue of recipient certification and collection access is addressed in detail in Section IV of this report.

At a minimum, a sample of the requested strain should be recovered and grown to demonstrate the strain remains viable and uncontaminated. Optionally the strain may also be tested to confirm it is correctly identified. Once complete, an unopened sample is prepared for dispatch.

The development of molecular biology has led to an alternative kind of sample which may be distributed. In certain common scenarios, recipients only require nucleic acids (usually DNA) and not the complete living organism. Certain culture collections currently offer purified nucleic acids as well as complete organisms, an option which involves little additional cost and / or labour to the collection. As pathogen DNA or RNA is a much safer material to handle and ship, this service may prove very useful to both strain recipients and pathogen collections such as those planned.

Documentation included with the sample must contain the information made available publicly from the database (see Section X, (B) Key Strain Information.) This information includes the taxonomic affiliation of the sample, the isolate's key



characteristics, suggested growth and storage conditions, and any threats or risks associated with the strain.

Detailed records of isolate distribution are required. At a minimum, records should include the name and location of the recipient, the nature of what was shipped, the method and date of shipping. The literature suggests the collection should retain a complete copy of whatever strain information and culture instructions accompany the sample. This precaution is a sensible one, as a strain's suggested procedures and protocols may change. Retaining a copy of strain documentation ensures that should issues arise, the collection can retrieve and confirm what documentation was sent.

## VII - Manpower

Collection staff represents a key ongoing cost for a Canadian pathogen culture collection. Estimating staff requirements was a three step process :

- 1) identify typical employee skill sets.
- 2) identify special training requirements and sources.
- 3) estimate total manpower required for each of the employee types identified in step 1.

Staff requirements were determined by study of collection literature, and comparison with other culture collections world-wide. This particular aspect of culture collection operation is very well documented. Each different group of organisms (bacteria, viruses, fungi) was addressed separately. In reality, this division is something of an oversimplification. While the fundamental biological differences between groups of organisms are such that scientists cannot be expected to work with more than one group, some support staff can no doubt be employed managing several types of organisms.

### 1) Employee Skill Sets

Several categories of employees will be required by any culture collection. These include:

- a) researcher / scientist / collection curator (PhD level training)
- b) technicians (BSc or technical certificate level training)
- c) database / IT administrators
- d) administrative personnel

As collection scientists identify unknown isolates and confirm the identification of collection strains, taxonomy and taxonomic classification are their key specialization. A background in taxonomy, especially molecular taxonomy / bio-informatics, should be a hiring requirement. Experience in specimen preservation would be useful, but may instead be provided through training. Each organism type (bacteria, viruses, fungi) will require at least one dedicated scientist to act as a collection curator and to provide expert identification and research guidance. Finding or training a specialist who can adequately cover two or more organism types is unlikely.

Technicians would conduct most of the day to day lab work within the collection, culturing organisms, conducting colony / plaque counts, preparing media, and maintaining the preserved samples. Technical staff should be trained as biology, microbiology, or medical microbiology technicians. Alternatively, a microbiology BSc. graduate would likely be adequate for work with bacteria and / or viruses, while a mycology / plant pathology BSc degree may be adequate training for a technician working with fungi. As many of the tasks assigned to technical personnel will be relatively unspecialized, technicians can likely be shared between organism types.

Any culture collection will require extensive information technology / database support, preferably via a dedicated database / IT specialist. Database / IT manpower issues will be addressed in detail in the Database / IT segment of the report.

Administrators include office staff, as well as pure administrators. No special technical skills are expected for these employees. Smaller collections will likely not require any dedicated administrative staff.

## 2) Special Training Requirements and Sources

Additional training may prove useful for new culture collection staff, in particular scientists and technicians. Administration and database / IT staff will likely not require any significant specialized training.

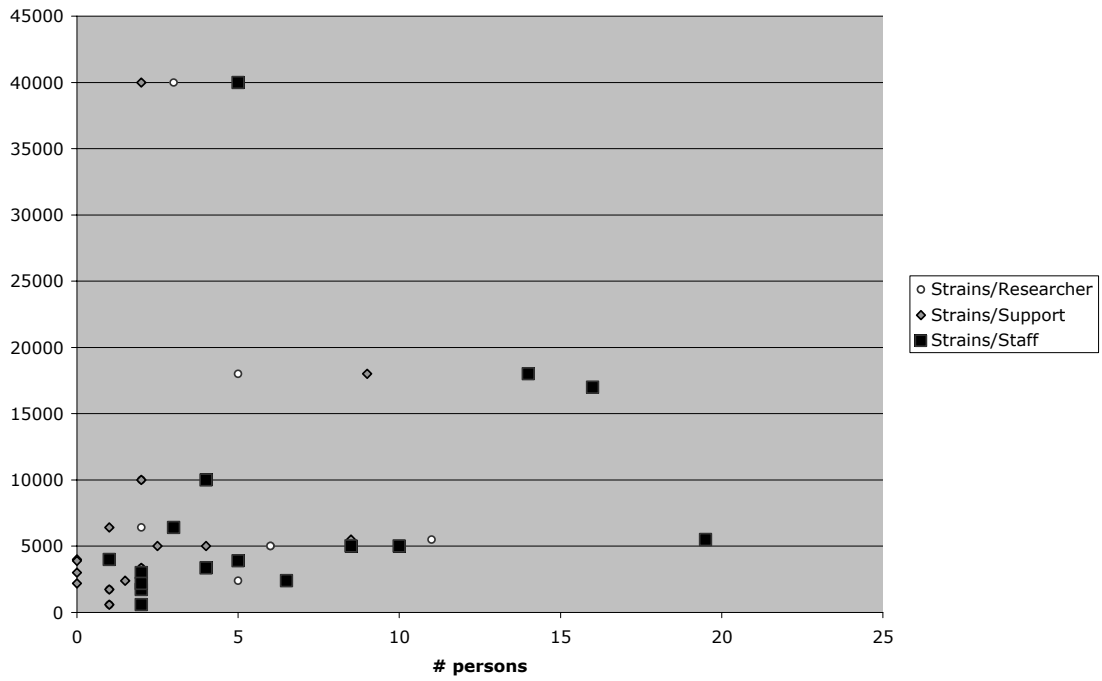
Routine culture collection techniques such as preservation and management of microorganisms are quite specialized and are not typically included within conventional academic training. The scientist / curator's crucial organism identification and taxonomy skills may also benefit from instruction. Fortunately, training in these topics is often available from culture collections themselves. Courses range from several days to weeks, and are typically tailored to the needs of the students. Costs range from \$200 - \$500 a day, or \$200 - \$4,000 a course. Many culture collections also offer training on a more informal basis. Appendix V lists a sample of training sources identified during the survey. Note in particular the May 3-7, 2004 curator training session offered by the World Federation of Culture Collections.

On this basis, training is budgeted at \$10,000 per new staff member (courses, travel and associated costs.). Training for technical staff may be significantly lower.

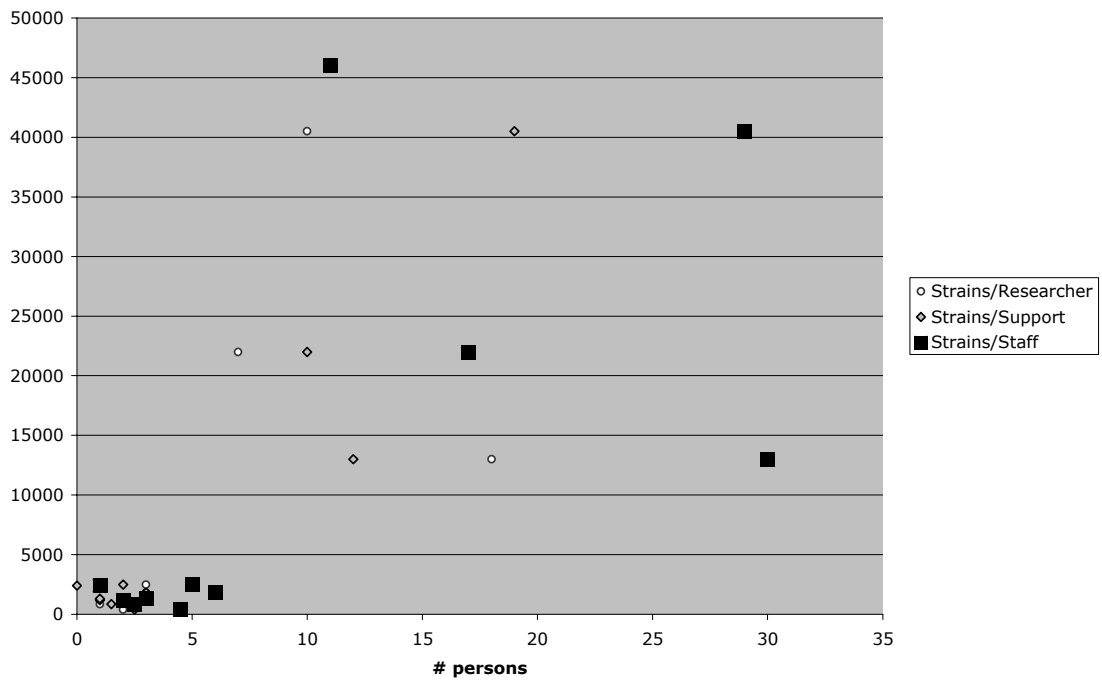
## 3) Manpower Estimate

Hypothetical culture collection staff requirements were individually calculated for bacteria, viruses, and fungi. In each case, the ratio of staff (scientists, technicians, and total staff) to strains was determined for a number of culture collections (tabulated in Appendix VI). When this data was plotted (see graphs 1, 2 and 3), values were widely scattered but showed the expected increase in staff requirements as the number of strains increased. Mean and median values are presented in Table 15. As expected, bacteria require the least staff, while fungal cultures are only somewhat more labour intensive. The typical staff required for virus cultures is much higher, unsurprisingly as these parasitic organisms are difficult to grow, purify, and quantify. The median value was chosen for projected staff requirements.

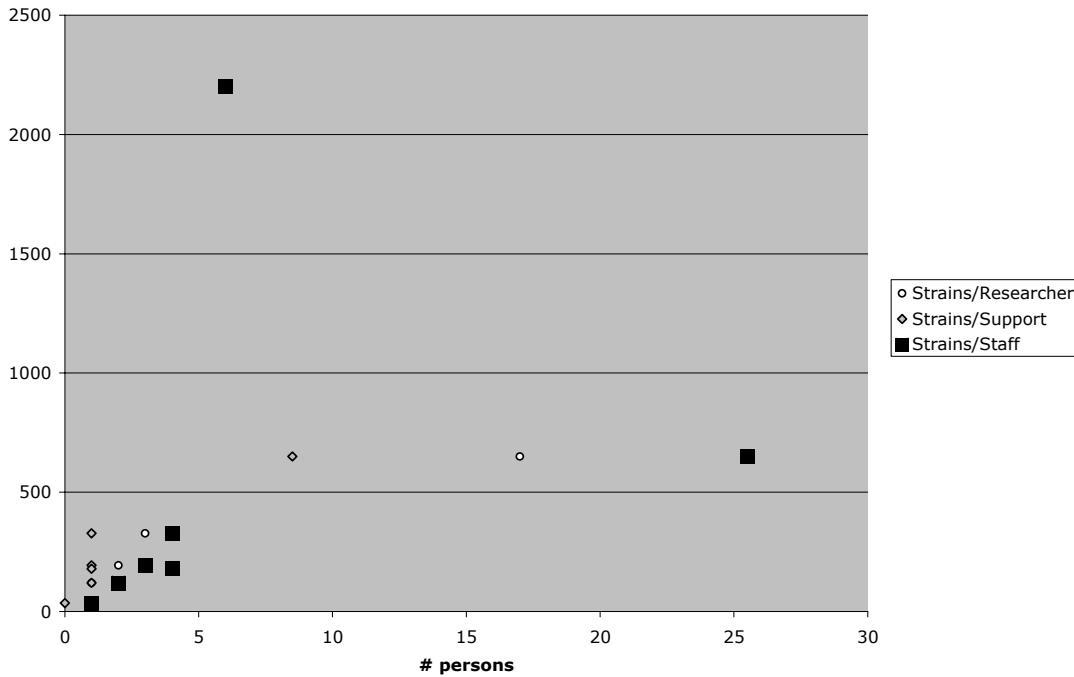
**Graph 1 - Bacterial Strains / Staff**



**Graph 2 - Fungal Strains / Staff**



**Graph 3 - Virus Strains / Staff**



**Table 15 Strain / Organism Type Manpower Requirements**

	Mean	Median
Bacteria / Researcher	2514	1300
Bacteria / Support	3899	1725
Bacteria / Staff	1631	963
Fungi / Researcher	1712	850
Fungi / Support	1166	1200
Fungi / Staff	1088	500
Viruses / Researcher	118	97
Viruses / Support	180	180
Viruses / Staff	97	60
Ratio Researcher / Support	Bacteria	2.03
	Fungi	1.13
	Virus	2.64

Table 16 Summarizes the manpower estimate amounts. The number of strains for the CBBC and CPC models represents either the number of known pathogen species (CBBC1 and CPC1), or the number of strains of pathogen species identified in culture collections (CBBC2 and CPC2). The 'general' category was added as a comparison

reference and represents a pathogen collection including all organisms, pathogenic or not, from genera containing known human pathogens. The CMCN strain holdings represent a virus CPC1, a bacterial CPC2, and the fungal pathogen held by the UAMC. This combination was chosen as representative of one possible CMCN configuration; in the author's opinion, a particularly efficient initial structure.

Staff requirements were calculated using the values found in Table 15 and the cost / complexity adjusted strain figures from Tables 5 to 12. As only bacterial, viral, or fungal specialists can serve as research scientists, any 'fractional' scientist was counted as a whole number. Technical staff, having both more general tasks and training, were presumed shared between the three organism types. Total staff numbers assume minimal administration personnel, as shown.

**Table 16 Collection Manpower Requirements**

Proposed Collection		CBBC 1	CBBC 2	CPC 1	CPC 2	General	CMCN
Number of Strains	Bacteria	38	500	221	2500	10000	2500
	Virus	61	100	160	450	750	160
	Fungi	3	100	102	2000	5000	1100
Scientists	Bacteria	0.08	0.77	0.28	2.31	9.23	2.3
	Virus	1.22	1.55	2.39	5.15	7.73	2.4
	Fungi	0.01	0.24	0.12	2.35	5.88	1
Technical Staff	Bacteria	0.06	0.58	0.21	1.74	6.96	1.7
	Virus	0.66	0.83	1.29	2.78	4.17	1.3
	Fungi	0.00	0.17	0.09	1.67	4.17	1
Total Lab Staff Requirements	Bacteria	0.10	1.04	0.38	3.12	12.47	4
	Virus	1.97	2.50	3.87	8.33	12.50	4
	Fungi	0.01	0.40	0.21	4.00	10.00	2
Database / IT		1	1	1	1	1	1
Administration							2
<b>Total Staff</b>		<b>5</b>	<b>7</b>	<b>8</b>	<b>16</b>	<b>39</b>	<b>13</b>
<b>Strains / Staff</b>		<b>20.4</b>	<b>100</b>	<b>60.4</b>	<b>309.4</b>	<b>403.8</b>	<b>289</b>

Unlike other staff numbers, the CMCN fungal staff (1 researcher, 1 technician) represent new UAMC staff in addition to the current independently funded UAMC staff (1 researcher, 1.5 technicians). The additional UAMC employees bring staff to a level which the collection curator (Dr. Lynn Sigler) believes would maximize collection efficiency and allow implementation of molecular biology / DNA sequencing procedures.

The "Strains / Staff" line offers some estimate of overall 'efficiency' of manpower use. Generally, large collections are more efficient than smaller collections. The smaller collections (CBBC1 and CPC1) are particularly inefficient as the bacterial and fungal collections do not provide an adequate workload for a dedicated scientist, or technician. The excellent CMCN efficiency is partially due to the integration of a pre-existing collection (the UAMC) whose current staff is not included in this estimate. Note that while the UAMC collection actually holds approximately 10,000 strains, only the

pathogens were included in these calculations. Had all UAMC holdings been included, the strain / staff ratio is 974. The large manpower size of the 'general' pathogen collection illustrates how, at least initially, a collection of this size is impractical. Given the initial collections will likely be configured as labs within a larger establishment, the CPC options appear suitable, especially the CPC1 viral, CPC2 bacterial and CPC2 fungal options.

Each scientist was projected to receive a salary of \$70,000 (DS4 level pay) and each technician \$50,000 (EG4 level pay) when making budget calculations. The database / IT employee salaries was estimated at \$60,000, and administrative staff at \$50,000

In all likelihood, the staff estimate used here is probably somewhat generous. Many long-standing culture collections maintain far more strains than the median or mean figure would suggest is possible. However, inadequate staff is a chronic culture collection problem, so these collections are likely not operating optimally.

## VIII - Capital Equipment Requirements:

Hardware required for collection operations represents the main source of culture collections capital costs. The capital cost estimate presented here includes a number of assumptions. The crucial assumption is any collection will be designed and implemented as a part of a larger institution, such as the NML or DRDCS. The rationale for this assumption is two-fold:

- 1) In even their largest forms, the proposed collections are relatively small (see the manpower estimate, section VII.) As a consequence, creating an independent institution would result in unacceptably high proportion of resources and budget being diverted from research and collection staff to support services and administration. Collections housed within a larger institution would use pre-existing support and administration resources, a far more efficient arrangement as the effective 'load' they add to the institution as a whole is relatively minimal. Individually, each of the CPC1 virus, CPC2 bacteria and CPC2 fungi collections could easily be housed in one or two conventionally sized 'lab rooms'.
- 2) As the culture collections are pathogen-oriented, the bacterial collection will include a significant number of BL level 3 containment organisms, and the viral collection a large number of both BL 3 and 4 organisms. As a consequence, these collections absolutely require sophisticated containment suites. The cost of these facilities is very high; DRDCS has recently purchased a BL 3 suite for over 1.2 million dollars. BL 4 facilities are far more costly; at the current time only one exists in Canada (the NML in Winnipeg). As a consequence, the proposed pathogen collections are only practical if configured within an institution which already possesses a BL 3 and / or BL 4 lab area.

This assumption is supported by a global culture collections survey which has shown that the early trend to large, free-standing institutional collections such as the ATCC has now been reversed (33). Instead, the majority of national collections are at least partially dispersed, and typically found associated with government labs, medical facilities, universities, and large businesses.

A standard suite of culture collection equipment was determined by surveying world culture collection resources (World Data Centre for Microorganisms) (34), from literature resources (see Appendix II), and during consultation with individual culture collections. The needs of different organism types are somewhat different, as tabulated below. The hardware required to implement a nucleic acid based microorganism identification system (discussed in section VI) was included as a part of the equipment requirements, though as a separate heading in the detailed cost breakdown.

As discussed previously, a Canadian pathogen collection can be assumed to be associated with or part of a larger organization. As a consequence, many of the resources required by a culture collection will already be present within the 'host' institution. Examples of these resources include DNA sequencers, transmission electron microscopes, and large capacity autoclaves. These often expensive resources are rarely



used to full capacity, and as a consequence should be available to the culture collection when needed.

An additional set of equipment will be required by any culture collection for day to day in-lab activities. Consequentially, a new culture collection will likely need to purchase these resources. Given the specialized nature of culture collection operations, additional, less frequently used equipment may also prove an absolute requirement. Therefore, equipment requirements can be divided into two groups: those required by the collection, but likely provided by the 'host' institution, and those which must be purchased for the collection itself.

A number of additional assumptions were made concerning the collection and its arrangements when making the equipment estimate:

- 1) Lab Organization : Due to their small size (staff and strains held), the CBBC1 and CBBC2 model collections were assumed to share a single set of labs. As a result, dedicated culture collection equipment was assumed shared among the three collections. The CPC1 and CPC2 collection equipment costs were calculated assuming all three organism types (bacteria, viruses, fungi) were held in separate labs and thus had their own equipment. The CMCN configuration is the same one used during the manpower estimate (Section VII) and includes the UAMC fungal pathogen holdings along with independent bacterial CPC2 and viral CPC1 collections.
- 2) Number of Duplicate Isolates Stored : The actual number of vials held in a collection was an important consideration when calculating equipment requirements (freezer space, liquid nitrogen storage requirements, cold rooms, etc.) For bacteria and fungi, 10 different vials / ampoules were assumed stored per strain. As viruses are so much more difficult and expensive to produce, 100 vials / ampoules were assumed stored per virus. If a storage system was not rated for the number of vials stored, each cubic foot was assumed to store 3000 vials. At least 50% additional storage capacity was allocated for each storage method.
- 3) Preservation Methods Used : Ideally, two different preservation / storage methods should be used. For the equipment plan, viruses were presumed stored (lyophilized or in solution) at both  $-86^{\circ}\text{C}$  and in liquid nitrogen vapour. Bacteria and fungi should be stored both by cryopreservation in liquid nitrogen vapour and in lyophiles (frozen or  $4^{\circ}\text{C}$ )
- 4) Backup Facility : A simple backup facility containing each strain was planned to safeguard collections in the event of a service interruption. This facility was projected to store only a small number of vials / ampoules per organism (one or two) when determining capacity.
- 5) Building / Room Space and Renovations : These cost estimates do not include the cost of the collection's physical space and associated work-area nor renovation costs.

Equipment costs were determined by searching scientific supplier catalogs and product lists. Some sample prices and suppliers are found in Appendix XI. Along with culture collection strain lists and procedures, this data was used to construct a detailed breakdown of equipment costs tabulated in Appendix XII. The capital laboratory requirements for the three proposed culture collections are summarized in Table 17:

**Table 17 Culture Collection Equipment Summary**

	organism type	Strains	Costs Required By Collection	Costs From Host Instit.	Total	Cost / Strain
CBBC1	all types	102	<b>\$259,000</b>	\$1,207,000	\$1,466,000	<b>\$2,539</b>
CBBC2	all types	700	<b>\$267,000</b>	\$1,207,000	\$1,474,000	<b>\$381</b>
CPC1	bacteria	221	\$238,000	\$1,057,000	\$1,295,000	\$1,077
	virus	160	\$240,000	\$1,207,000	\$1,447,000	\$1,500
	fungi	102	\$256,000	\$1,207,000	\$1,463,000	\$2,510
	all types	483	<b>\$734,000</b>	\$3,471,000	\$4,205,000	<b>\$1,520</b>
CPC2	bacteria	2500	\$285,000	\$1,057,000	\$1,342,000	\$114
	virus	450	\$279,000	\$1,207,000	\$1,486,000	\$620
	fungi	2000	\$261,000	\$1,207,000	\$1,468,000	\$131
	all types	4950	<b>\$825,000</b>	\$3,471,000	\$4,296,000	<b>\$167</b>
CMCN	bacteria	2500	\$285,000	\$1,057,000	\$1,342,000	\$114
	virus	160	\$240,000	\$1,207,000	\$1,447,000	\$1500
	fungi	1100	\$280,000	-	\$280,000	\$255
	all types	3760	<b>\$805,000</b>	\$2,264,000	\$2,919,000	<b>\$214</b>
Backup Facility						\$51,000

Key figures are in bold type. The 'Costs Required by Collection' column indicates the cost of equipping the culture collection labs themselves. The 'Costs from Host Instit.' column represents the cost of equipment already present in the host institution. Much of the equipment provided by the host institution is involved in DNA sequencing and analysis, a key tool with high culture collection utility (see Section VI).

The 'Total' column is the sum of the preceding two values. The advantage in locating any culture collection in a host institution is quite obvious. Note that in each, the overall difference in equipment costs between culture collection 'labs' is not all that great (\$238,000 to \$285,000), regardless of the number of specimens the collection housed.

The CMCN 'fungi' data represents the UAMC. Once again, of the 10,000 strains held by the UAMC, only the 1100 pathogen strains have been used in these calculations. The \$280,000 capital cost was obtained by consultation with the collection curator: \$30,000 for basic molecular biology apparatus, and \$250,000 for facility expansion and renovations.

The Cost / Strain values indicate the overall 'efficiency' of a culture collection. As found during the manpower analysis, smaller collections are less cost effective. Virus collections have much higher cost per strains principally due to the limited number of pathogenic viruses housed in the various proposed collections. Both the CPC2 and

CMCN options are approximately equal in cost efficiency when assessing pathogens, note that if the entire UAMH collection is added, the CMCN equipment cost per strain is reduced to \$63.

Several additional factors may reduce capital costs. Much of the equipment (lyophilizers, incubators, biosafety hoods, etc) used in culture collections has become fairly well standardized over the past several decades. As a result, the most modern apparatus is not an absolute requirement. Older equipment already owned by a host institution is likely more than adequate for a new culture collection. Curators are encouraged to investigate whether otherwise surplus equipment may be available, perhaps following a minimum of repair and reconditioning. In the author's experience, this kind of surplus equipment is often available in larger research organizations.

A point of interest, while surveying culture collection equipment requirements, lyophilizer systems produced by Edwards, a UK firm, were repeatedly preferred. Unfortunately, this firm no longer appears to be in business. Nevertheless, Edwards lyophilizers are available via online second-hand scientific equipment suppliers, a route which may be worth investigating. During the capital cost analysis, the author repeatedly found these equipment suppliers sold many items used in culture collections at significantly reduced prices.

## **XI - Ongoing Requirements:**

Ongoing requirements are those costs routinely accrued by a culture collection during day to day operations. These costs do not include capital equipment purchases and employee salaries. Instead, these costs include largely 'disposable' supplies: culture media, disposable sterile supplies, liquid nitrogen, and the myriad other items a biology lab purchases and consumes. As the projected culture collections plan to use molecular biology as a tool, these supplies would include disposables used during DNA acquisition and analysis, along with PCR and DNA sequencing supplies.

Determining ongoing requirement costs was originally planned to parallel the collection manpower estimate. The budgets of a large number of collections would be plotted against the number of strains they contain, keeping individual organism types (bacteria, viruses, fungi) separate. Unfortunately, this analysis proved impossible due to a number of unforeseen complications:

- 1) **Published Budgets** : unlike staffing, very few collections publicize their operating budgets. Direct inquiries to collection curators were rarely helpful. They themselves were often unable to provide even ballpark figures.
- 2) **Collection Holdings** : larger culture collections such as the ATCC do often publish an overall annual budget, but these collections often contain a complex mix of sample types. Unfortunately, one cannot assume that a collection spends 15% of its budget on the viruses which make up 15% of culture collection. Detailed analyses of which living resources require certain funds simply proved unavailable. Worse, even within an organism type, not all organism costs are roughly proportional. For example, a bacterial collection containing a well studied and easily managed species (eg. *E. coli* strains) may be very inexpensive to maintain, while another bacterial collection containing soil anaerobes may be many times as costly. The net result; extrapolations between a stated collection budget and its holdings are at best general.
- 3) **Collection Activities** : not all collections engage in the same routine activities. One collection may conduct exhaustive quality control procedures when processing and shipping isolates. Another may send ampoules without verifying the organism is correctly identified or even viable. This factor was often obscure until a more detailed assessment of culture collection services was conducted. Some very large and low-cost collections are quite literally holding services where individual isolates are rarely, if ever, monitored. Whether a culture collection uses molecular biology / DNA techniques may prove a very significant cost factor.
- 4) **Organism Type Rarity** : While many collections exist for certain types of organisms (eg. medical bacteria), other organisms are rarely collected. The general scarceness of human and animal virus collections is probably the best example of this scenario. Simply, there are so few of these holdings that any generalization is difficult.

- 5) National Differences : attempts to compare budgets among collections located outside Canada is difficult at best, given local material and supply costs vary widely. Ideally, data should have been drawn from Canadian, North American, and perhaps western European collections. The number of such collections is quite limited.
- 6) What Costs Does a Collection Cover : as noted previously in this report, many culture collections are associated with a 'host' organization. This arrangement seriously impedes attempts to draw general conclusions on collection costs. Some collections may pay in part or whole for accounting, custodial services, heating, electrical bills, and other costs, while other collections receive these services essentially 'for free.'

Estimating needs by simply 'totaling up' ongoing costs of a hypothetical collection were impossible. Guesstimating ongoing costs (the type of bacterial media a collection might need, disposable plates, gloves, electrical bills, liquid nitrogen, reagents etc.) is beyond the scope of this study, and simply futile, given the unique needs and complexity of any of the proposed culture collections.

With little accurate budgetary information available, only a very general estimate may be made of ongoing requirements. Consequentially, these values should be viewed with the utmost suspicion during collection planning. Based on the incomplete information available, maintaining each bacterial strain requires \$10 annually, each fungus \$20, and each virus \$300.

The projected ongoing costs per culture collection are listed below in Table 18. Note that unlike the other values, the \$60,000 CMCN fungal budget was not derived from the \$20 per strain calculation but instead represents an annual budget increase in addition to current NSERC funding (\$42,000). This supplement was recommended by the UAMH curator to allow implementation of DNA / molecular biology techniques.

**Table 18 Ongoing Costs**

	organism type	Strains	Costs Required By Collection	Total Collection Staff Salaries
CBBC1	bacteria	38	\$380	\$320,000
	viruses	61	\$18,300	
	fungi	3	\$60	
	all types	102	<b>\$18,740</b>	
CBBC2	bacteria	500	\$5,000	\$440,000
	virus	100	\$30,000	
	fungi	100	\$2,000	
	all types	700	<b>\$37,000</b>	
CPC1	bacteria	221	\$2,210	\$510,000
	virus	160	\$48,000	
	fungi	102	\$2,040	
	all types	483	<b>\$52,250</b>	
CPC2	bacteria	2500	\$25,000	\$1,010,000
	virus	450	\$135,000	
	fungi	2000	\$40,000	
	all types	4950	<b>\$200,000</b>	
CMCN	bacteria	2500	\$25,000	\$980,000
	virus	160	\$48,000	
	fungi	1100	\$60,000	
	all types	3760	<b>\$133,000</b>	

These values are, hopefully, an over-estimate of actual cost. Many Canadian culture collections maintain strains for far less. The Salmonella Genetic Stock Centre maintains over 10,000 strains with an annual budget of \$20,000 (\$2 / strain) (35). The UAMC holds over \$10,000 strains with an annual budget of \$42,000 (\$4 / strain). While no comparable culture collection exists in Canada, the UK National Pathogenic Virus Collection houses over 400 strains with an annual budget of \$80,000 (\$200 / strain).

Even should the cost per strain values be significantly in error, ongoing costs will likely represent only a small fraction of the proportion of annual culture collection expenses. Table 18 lists the estimated annual staff salaries as well as the ongoing costs. A comparison of the two figures shows that in any collection, staff costs are far larger than annual material supply costs. Consequentially, even significant underestimation of ongoing costs will result in only a small increase in the overall annual collection budget.

## **X – Database / IT Support**

Any culture collection requires a mechanism which allows potential culture collection users to determine whether the strains they require exist within the culture collection. While this information was traditionally found in published catalogues, this approach is now effectively obsolete. Digitally storing the collection's "catalogue" would provide a far superior method for users to manage and retrieve information about collection holdings. This information becomes the collection database.

Unfortunately, world-wide culture collections consistently fall short of an acceptable standard when maintaining information databases. The degree to which this shortcoming cripples collection utility cannot be overstated. The ability of the public to use a collection in a meaningful manner is not simply limited by the ability to order or request a strain; the end-user must also know whether strains with the desired characteristics exist. Therefore, public information must provide all information needed to identify the required strain.

Fortunately, current information technologies allow efficient low-cost mechanisms for these requirements. Culture collection IT issues include :

- A) Information Accessibility : information concerning strains must be accessible to both public users and private users. Public users have read only privileges to select data while private users are free to add, edit, and manipulate collection data. With the collection's "catalogue" digitally stored in one location, no paper copies are necessary. Access to public information can be set up through a website with searchable forms. Private in-house information for management purposes can be accessed via a secure website, or better yet, the collection's own dedicated application with a secure direct line straight to the central data source. Appendix III illustrates a suggested public search-engine design, along with a sample output. Efficient webpage and search-engine design is an absolute requirement; a great many otherwise useful culture-collections are severely hampered by extremely poor search-engine design. If public users cannot identify the culture they desire, that culture is effectively beyond their reach.
  
- B) Key Strain Information : to identify a particular strain, public users require access to pertinent strain characteristic information. Culture holdings become vastly more useful by storing these key facts within the culture collection's database, and making those terms searchable through the collection's webpage. Key strain information includes many strain attributes (many of which are typically under-documented for the public.) Key strain information includes:
  - 1) taxonomic information (current and obsolete)
  - 2) historical information - such as the significance of strains, medical facts and history of pathogens
  - 3) genetic information (especially if unusual for the species / group)
  - 4) phenotypes (especially if biochemically significant, rare, dangerous, unusual for the species / group)

- 5) where the strain originated (source individual making the deposit, whether the strain was transferred from another culture collection. Researchers often identify strains by culture collection accession numbers, so source collection accession numbers are a very key reference)
- 6) where the strain is stored - particularly significant should a culture collection have several subdivisions
- 7) morphology - cell and culture, photographs may prove useful
- 8) hazardous features / characteristics - the threat level represented by any part of the collection must be clearly indicated at all times.

Of course, additional information will be stored but not available to the public. This additional information is necessary within the culture collection itself when tracking the culture viability, current stocks, and physical location. Examples of this in-house data may be found in Appendix IV. One open question is the degree of tracking detail necessary for individual samples. Current information technology can allow easy tracking by assigning individual vials unique number and barcode identifiers. For example, the ASTRO antigen/antibody database used by the Department of National Defence, CDC and USAMRID uses barcode technology. If barcode scanning is added to sample handling procedures, a significant decrease in errors such as sample misidentification should occur. Employing barcode technology would allow more accurate inventory control.

Populating the database with additional information (eg. growth media, storage conditions and methods, etc) may prove valuable when retrieved through the public search engine. Appendix IV describes the suggested fields within the culture collection database, along with some key comments concerning individual items.

Recording a wealth of strain attributes, associated facts, and strain history may seem a needless complication and expense, especially if the data does not directly relate to culture collection operations. In fact the opposite is true. The value of a particular organism is directly related to what is known about that organism. Making additional non-sensitive information available to the end-user increases the potential value of a particular strain.

Given this fact, a Canadian culture collection would also benefit from any additional information which could be "linked" to the holdings. An ever-growing amount of information is being collected online, and available for search and retrieval. This pool of organized information represents a potential mechanism to significantly increase the utility of a Canadian culture collection by means of reference. While the technology and standardization of current electronic sources may be inadequate to allow immediate exploitation, collection curators are encouraged to take advantage of these resources whenever the opportunity presents itself.

For example, referencing strains to a list of academic publications which address that particular isolate would be valuable to both public and private users. Increasingly, taxonomic and index information is being made available online, often by the very organizations responsible for creating standards and setting official nomenclature. An excellent example of just such a resource is the Universal Virus Database of the International Committee on the Taxonomy of Viruses (36). This webpage not only



maintains an index of current virus taxonomy but also provides an increasingly sophisticated 'encyclopedia' function. For example, see :

Smallpox virus : <http://ictvdb.bio2.edu/ICTVdB/58110001.htm>

Yellow Fever virus : <http://ictvdb.bio2.edu/ICTVdB/58110001.htm>

Unfortunately, most of these online web based resources are still in development, and may no doubt undergo significant alterations before reaching a mature form.

Nevertheless, initiatives exist to cross-reference biological information. Networks of strain information have been established in various areas; the European Common Access to Biological Resources and Information (CABRI) webpage (37) is a good example. The Microbial Strain Data Network (38) is a general United Nations initiative of this type. Many information services such as the Microbial Information Network Europe have yet to shift to web access. Living material held in Canadian culture collections would obtain increased accessibility should Canadian databases associate with these larger organizations. However, any such coordination must not be at the expense of providing service within Canada.

Referencing the collection information to other online resources could bring many benefits. Instead of allocating staff to redundant research, certain sources may be able to provide up-to-date and comprehensive taxonomic and biological information on organisms. Collection curators are strongly recommended to participate in processes which, ideally, will produce standard electronic sources for all types of biological information.

Finally, the database itself can be more than merely a method for parties to identify what organisms are stored within a collection. The database can also assist in reference and identification of unknown samples. By adding and referencing additional information the database itself may become a significant tool in pathogen identification. If government agencies involved in pathogen identification (agricultural and human) use the culture collection's database as a pipeline for established tests and pathogen identification standards, the culture collection can be both an information reference tool and the source of known biological standards. In this sense, a Canadian pathogen collection can provide a convenient and accessible unified national standard and reference.

### **Database / Information Technology Structure**

Unlike other components of a Canadian culture collection, the collection database / IT elements need not have any particular location, and therefore can be centralized. The scalability of a culture collection database is another unique feature of this resource. Once designed and constructed, a database may be used by many different collections with only minimal maintenance costs. As any culture collection will require a new dedicated database, the opportunity exists to aggressively exploit this resource. If a Canadian pathogen oriented culture collection is established, other Canadian culture collections could use and benefit from this new database.

The strategy of having more than one culture collection share a common database and web interface has been adopted by a number of national culture collections, including the Belgian Co-ordinated Collections of Micro-organisms (BCCM) and the United Kingdom National Culture Collection (UKNCC.) Merging database resources offers increased utility for all participants with minimal costs and complications. This fact is a key element of a CMCN strategy as discussed in Section IV of this report. As previously noted, the 1989 Task Force on the Status of Culture Collections in Canada report to the Minister of State (Science and Technology) (23) identified a common Canada-wide culture collection database as one of the key resources necessary for effective development and use of Canadian genetic resources. This possibility could easily be explored should Canada choose to develop a new set of national culture collections,

### Database / Information Technology Costs

Database / IT costs are summarized in Table 19, below:

**Table 19 Database / IT Costs**

Proposed Collection		CBBC 1	CBBC 2	CPC 1	CPC 2	CMCN
Startup Costs	Server	\$10,000	\$10,000	\$10,000	\$10,000	\$10,000
	Workstations	\$5,600	\$7,000	\$9,800	\$18,200	\$12,600
	Printers	\$1,000	\$1,000	\$3,000	\$3,000	\$3,000
	Application	\$100,000	\$100,000	\$100,000	\$100,000	\$100,000
	Webpage	\$10,000	\$10,000	\$10,000	\$10,000	\$10,000
	Total	\$126,600	\$128,000	\$132,800	\$141,200	\$135,600
Ongoing Costs		low	low	low	low	low

Database / IT costs can be split into two categories, those required when setting up the resource, and ongoing costs. Start-up costs in turn, can be divided into hardware and software. The network will require a server with associated database software, and each collection will require a number of workstation computers (one per scientist, one per every two technicians). Prices include barcode technology support and one barcode label printer per collection. The in-house application used by private users is expected to require a 6-9 month development period, while the public website should require 2-3 months.

Ongoing costs are principally personnel related. A single individual (the Technical Administrator) will be required for database maintenance. This individual would have the following responsibilities:

- Initial configuration of database for software
- Daily maintenance procedures, integrity checks
- Exporting/Importing; integrating foreign data
- 24/7 on-call
- Tech support for all nodes/facilities of the collection

This individual is capable of performing these tasks out of any of the collection's facilities or completely offsite. Hardware maintenance must be performed on-site but is not expected to require more attention than yearly check-ups.

Should the in-house application require attention, the original programmer will likely be required (estimate \$150 / hour for custom software support). However, this cost may be waived if the author of the software stays on staff as the Technical Administrator. Required updates, changes, and modifications could then be bundled into those responsibilities described in Database Maintenance.

## XI - Culture Sources

Naturally, the proposed Canadian culture collections will require sources for the biological resources they are to contain. During this survey, an assessment was attempted of what kinds of resources exist world-wide, along with the costs and feasibility of accessing these resources. As Canada has a large domestic medical fungal culture collection (the UAMH), no specific survey of fungal collections was made. No Canadian viral or bacterial analogs exists.

Absent from this analysis is an attempt to document resources already within Canada. An independent study by Health Canada is currently surveying domestic microbiological resources, focusing in particular, on those specimens found in provincial and Federal medical laboratories. These strains can provide a useful element of the original 'seed' stock for all the proposed pathogen collections. Some strains may be very unusual or unique, only isolated and / or preserved within Canada. These unique strains are very important for a number of reasons, not the least being that these strains simply are not available elsewhere. If these isolates appear uncontaminated and in good condition, collection curators are encouraged to use these domestic sources. Building a close working connection between Canadian medical labs and the proposed pathogen collections offers mutual benefit, particularly if new or significant pathogens are automatically added to the Canadian pathogen collections.

Traditionally, Canada has used the American ATCC general organism collection as its source for well characterized organisms. As discussed previously in Section I, this collection is no longer useful for pathogenic species. Furthermore, the ATCC is no longer a suitable source of non-pathogenic strains either. The ATCC Material Transfer Agreement includes the following clause :

**“The Purchaser shall not distribute, sell, lend or otherwise transfer the Material or Replicates for any reason.** Any commercial use of the Material, Replicates, and Derivatives is prohibited without ATCC's prior written authorization. Your use of the Materials may require a license from a third party or be subject to third party restrictions ("Third Party Terms"), which you may learn about in the catalog description for the particular Material and which are either set forth below or available at [www.atcc.org](http://www.atcc.org). If there is a conflict between this MTA and Third Party Terms, the latter shall govern.” (emphasis added.)

Fortunately, this clause appears quite exceptional among culture collections. No parallels were noted in other culture collection transfer agreements; indeed the underlying philosophy of the culture collection community is to share and thereby preserve resources, not hoard material and maximize profits.

No significant alternative public source within the US was identified, and direct transfer of material from US government agencies seems unlikely given current US export / import restrictions.

As a result, Canada must turn to other foreign sources. A wide variety of culture collections exist world-wide, and almost all will distribute the samples they hold. The WFCC-MIRCEN World Data Centre for Microorganisms (WDCM) maintains a web based list of world culture collections (39, 40). This resource is very useful when

searching among culture collections for general organism types, and determining contact information, services, and fees. Unfortunately, WDCM strain catalogs are all but impossible to use on those occasions when they are present. As noted previously within this report, animal / human viral culture collections are very rare, though many pathogenic bacteria and general bacteria culture collections exist.

A variety of possible culture collection sources are listed in Appendix XIV. A number of particularly interesting sources are worthy of some additional discussion. First, the various collections making up the UKNCC appear to be an excellent source for both bacteria and viruses. The National Collection of Type Culture (NCTC) holds both pathogenic and non-pathogenic bacteria. This collection was one of those used to generate pathogen strain lists in section V of this report, and held 71% of the pathogen list (the ATCC held 85%). The National Collection of Pathogenic Viruses (NCPV) is a new virus-only collection, in operation only three years. Nevertheless, the NCPV houses one of the most extensive public virus collections and is actively expanding their public holdings. This collection contains many viruses (including exotic, high BL viruses) in addition to those listed in the catalog. Viruses are being added to the public catalog as they are fully characterized and have their identify and purity confirmed. UKNCC members offer an additional advantage, as these collections have adopted very stringent quality control standards. Contaminated or misidentified samples are a significant issue in the culture collection community; some major collections (the ATCC in particular) possess a very poor reputation. As any new culture collection will encounter many challenges, minimizing the likelihood of any new acquisition being misidentified, contaminated, or dead is a significant advantage. Overall, these UK collections appear to be excellent candidates as collection strain sources.

In the event UK sources alone prove inadequate, a wide variety of bacterial collection alternatives exist. Some are listed in Appendix XIV, others may be found at the WDCM website. Animal / human virus source options are far more limited. Both the Czech National Collection of Type Culture and Czech Animal Pathogens Institute have significant collections, as does the German Institute of Virology. No recent catalogs from these collections were located, nor do they seem to maintain an online catalog. A particularly large but poorly documented animal virus collection is apparently held by the Norway Medical Microbiological Laboratory. Again, no printed or online catalog could be identified, however if the information at the WDCM is correct, the stated size of the collection (1880 strains) is amongst the world's largest.

Most culture collections charge a small fee for distributing samples. No significant fee difference was noted between different kinds of organisms (bacteria, viruses, fungi.) Fee schedules are often different for academic, government, commercial, and medical users. Typically, fees are significantly higher for businesses. Prices range from \$50 to \$200 an isolate, typically costs charged government, medical and academic users are about \$75 per strain, though in certain cases no charge is made. Bulk transfers are often given a discount.

Only rarely does one find a distinct fee charged for isolates being sent to another culture collection. Instead, the majority of culture collections prefer to trade isolates rather than demand payment. These 'trade agreements' may be more than one time exchanges, many collections develop long-standing links in which new acquisitions are sent to trading partners once the strain have been identified, screened for contamination,

characterized, and stored. A new Canadian culture collection would clearly benefit from entering into these kinds of agreements. Obviously, trading strains requires Canada have unique or unusual biological resources to exchange, thus the value of the unique domestic holdings mentioned earlier. For these trade agreements to be attractive, the material offered by Canadian collections must be of high quality. Once again, collection expertise is necessary to provide accurate, high-quality samples which can then be offered in exchange.

It is unlikely that new Canadian pathogen culture collections will have to purchase all the strains required. However, for budgetary purposes, this assumption has been made. Each isolate is assumed to cost \$75, along with \$25 for shipping. Making any estimate of the cost of actually ‘adding’ a strain to a collection (stock acquisition procedure, see section VI) is difficult; some organisms will require little characterization, some a great deal. However, isolates from other culture collections should have well established maintenance and storage protocols and thus not incur additional costs.

Using these assumptions, Table 20 below shows the costs to purchase and deliver strains to make up the various proposed culture collections.

**Table 20 Strain Acquisition Costs**

Proposed Collection		CBBC 1	CBBC 2	CPC 1	CPC 2	CMCN
Number of Strains	Bacteria	38	500	221	2500	2500
	Virus	61	100	160	450	160
	Fungi	3	100	102	2000	0
	Total	102	700	483	4950	2660
Cost		\$10,200	\$70,000	\$48,300	\$495,000	\$266,000

As at least some of the strains will already exist within Canada, and that other strains will be acquired in trade or for free, these figures should overestimate the actual cost of stocking any of the proposed collections.

## **XII – Total Costs and Implications**

The estimated cost of the various hypothetical culture collections is shown below in Table 21. The “general” collection discussed previously has been included for comparison. The two CMCN columns indicate efficiency when including the 1,100 pathogenic fungal strains held by the UAMC (CMCN (path.)) or the entire 10,000 UAMC isolate collection (CMCN (total)).

As noted throughout the report, the author has intentionally attempted to err on the generous side when making cost, manpower, and requirement estimates. These figures also do not account for potential collection income obtained through training, identification services, and isolate distribution. In essence, these values are a worst-case scenario.

Cost per strain, and manpower per strain values can be used as a method to measure collection efficiency. Obviously, larger collections are more efficient than smaller collections.

The CBBC, CPC and CMCN collection components are all small enough that they may be located within a host institution. Consequentially, equipment costs are quite low. The hypothetical general collection has no such advantage; this many staff could not be added to any currently existing medical / BW facility. Instead this collection size and staff are within the scale of ‘institutional’ collections such as the ATCC and the CBS and therefore would require a complete equipment set. The general collection would likely require still more capital commitments including a new or extensively renovated building, and complete BL 3 and BL 4 suites. Conservatively, these additional costs would likely exceed 10 million dollars.

Some conclusions can be drawn. First, manpower will likely represent the single largest cost, both in setting up and operating any collection. Second, larger collections are intrinsically more efficient provided they can draw equipment and facility support from a larger host institution. Last, a distributed culture collection network is at least as efficient as a single unified culture collection.

**Table 21 Overall Collection Cost / Manpower Analysis**

Proposed Collection Number of Strains	CBBC 1	CBBC 2	CPC 1	CPC 2	CMCN (path.)	CMCN (total)	General
Bacteria	38	500	221	2500	2500	2500	10000
Virus	61	100	160	450	160	160	750
Fungi	3	100	102	2000	1100	10000	5000
<b>Total</b>	<b>102</b>	<b>700</b>	<b>483</b>	<b>4950</b>	<b>3760</b>	<b>12660</b>	<b>15750</b>
Total Collection Staff	5	7	8	16	13	13	39
Strains / Staff Member	20.4	100.0	60.4	309.4	289.2	973.8	403.8
<b>Startup Costs</b>							
Collection Equipment	\$259,000	\$267,000	\$734,000	\$825,000	\$805,000	\$805,000	\$2,000,000
Backup Facility	\$51,000	\$51,000	\$51,000	\$51,000	\$51,000	\$51,000	\$100,000
Staff Training	\$50,000	\$70,000	\$80,000	\$160,000	\$130,000	\$130,000	\$390,000
Isolate Acquisition	\$10,200	\$70,000	\$48,300	\$495,000	\$266,000	\$266,000	\$1,575,000
Database / IT	\$126,600	\$128,000	\$132,800	\$141,200	\$135,600	\$135,600	\$434,000
Building / BL 3 and 4	\$0	\$0	\$0	\$0	\$0	\$0	\$10,000,000
<b>Total / Strain</b>	<b>\$496,800</b>	<b>\$586,000</b>	<b>\$1,046,100</b>	<b>\$1,672,200</b>	<b>\$1,387,600</b>	<b>\$1,387,600</b>	<b>\$14,499,000</b>
Staff Salaries	\$320,000	\$440,000	\$510,000	\$338	\$369	\$110	\$921
Ongoing Requirements	\$18,740	\$37,000	\$52,250	\$980,000	\$980,000	\$980,000	\$2,420,000
Database / IT	low	low	low	low	low	low	low
<b>Total / Strain</b>	<b>\$338,740</b>	<b>\$477,000</b>	<b>\$562,250</b>	<b>\$1,210,000</b>	<b>\$1,113,000</b>	<b>\$1,113,000</b>	<b>\$2,845,000</b>
<b>Total / Strain</b>	<b>\$3,321</b>	<b>\$681</b>	<b>\$1,164</b>	<b>\$244</b>	<b>\$296</b>	<b>\$88</b>	<b>\$181</b>



## **XIII - Conclusions**

In addition to the cost figures presented in Section XII, a number of broad conclusions can be drawn from this report:

- 1) Canada requires a pathogen culture collection for medical and BW needs, but would benefit from additional culture collection resources.
- 2) To be useful, a collection must be comprehensive, stable, accurate, and accessible.
- 3) A pathogen collection must contain bacteria, viruses and fungi. Protozoa are not a requirement.
- 4) Collections may be independent or a component of a larger culture collection network. A collection network is more accessible, stable, and can include and assist pre-existing living resources. A collection network is cost effective and promises to correct long-standing deficiencies in Canada's culture collection strategy.
- 5) A comprehensive pathogen set may be housed within a reasonably sized collection. In the UAMC, Canada already possesses a large fungal pathogen resource.
- 6) Collection procedures and equipment must ensure strains are correctly identified, uncontaminated, and viable. Failing to meet these criteria results in collections which are unstable, inaccurate, and thus useless.
- 7) Expanding culture collection procedures to incorporate molecular biology techniques offers great advantages in the present and future, at moderate expense.
- 8) Mid-sized collections or a collection network require a modest sized staff. Small collections make inefficient use of manpower.
- 9) Provided culture collections are associated with a 'host' institution, capital equipment requirements are modest. A 'free-standing' collection is not feasible, efficient, or necessary.
- 10) While the annual cost of supplies and materials is difficult to estimate with precision, these costs should be a small fraction of annual budgets.
- 11) Sophisticated and efficient information technology / database resources are required for a useful and accessible collection. Detailed strain descriptions make the strains themselves useful to all users.
- 12) Database / IT resources may be shared among culture collections with minimal additional expense.

- 13) The US is no longer a viable source for biological material, but UK sources likely represent an adequate alternative. Additional international sources may prove necessary, especially for viruses.
- 14) Canada can afford to develop a national pathogen collection. This collection would be best organized within a network of new and pre-existing Canadian culture collections.

Throughout this report, various possible culture collections have been described as distinct alternatives. Realistically, these model collections are simply points on a continuum of different sized culture collections. Collections will grow along this range over time.

Thus the model collections represent key positions on this range. The two CBBC collections are intended to do much the same task. The same is true of the CPC1, CPC2 and CMCN collections. In reality, any collection would likely fall between these hypothetical scenarios. A new CPC collection would start near the CPC1 model, and likely grow into a CPC2. The clearly superior efficiency of the larger model collections indicates how large a collection should be – as large as can be managed. Nevertheless, attempting to create and populate a large collection from scratch is probably unwise. Rather, these resources will have a natural and progressive pattern of growth as expertise and materials accumulate. This growth should be encouraged, not only to obtain increased efficiency, but simply because the living microbiological materials themselves are useful.

The CMCN collection is a special case, and deserves additional commentary. The CMCN proposed is in itself the ‘small’ end of the culture collection network range. Throughout this report, the cost efficiency of the CMCN model has been examined both in regards to Canada’s national pathogen library, and total CMCN holdings, pathogenic or not.. If the sole concern is building a national pathogen collection, the CMCN option is somewhat less costly than the CPC2 collection, and equally efficient. When analysis expands to consider Canada’s biological resources as a whole, the CMCN is the vastly superior scientific and financial alternative. The proposed CMCN collections and organization is a seed from which a national culture collection strategy can develop. As member collections grow and additional collections join the network, overall CMCN efficiency improves.

If Canada is to possess collections of pathogens, something like the bacterial and viral CPC collections must be established. Those collections can be established and operate as islands, or within a larger national strategy. Regardless of which path is chosen, the overall costs are similar. Nevertheless, the CMCN alternative offers far higher future potential and efficiency.

But efficiency should not be the sole concern when addressing living resources such as these. The collections proposed are pathogen collections, first and foremost. There is no denying Canada’s need to hold pathogen stocks. However, there is no need to limit Canada’s collections to the role of pathogen storage, and pathogen storage alone.

Culture collections are literally libraries, archives, museums. Their contents have an absolute scientific, industrial, medical, and intrinsic value. Consequentially, what can

be saved should be saved. In this regard, Canada's performance has been, at best, negligent. This should end. A current and pressing need exists, but fulfilling this need should not be the end of the process. Rather, just as the people and the state place value in our natural landscape, our literature, and our history, all living things too have value. By planning and implementing a solid, well-considered microorganism culture collection strategy, we can start the process to create a literally priceless resource, for Canada and the world.

## References:

- (1) <http://www.atcc.org/Services/SafeDep.cfm>
- (2) <http://www.ukncc.co.uk/html/Information/preservation.htm>
- (3) Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and Regulations, WIPO Publication Number 277, 1977, Geneva
- (4) <http://www.wipo.org/treaties/registration/budapest/>
- (5) Convention on Biological Diversity, Secretariat of the Convention on Biological Diversity, <http://www.biodiv.org/doc/legal/cbd-en.pdf>
- (6) World Resources Institute, The World Conservation Union, United Nations Environment Programme, Food and Agriculture Organization of the United Nations, United Nations Educational Scientific and Cultural Organization, (1992) *Global Biodiversity Strategy: Guidelines for Action to Save, Study, and Use Earth's Biotic Wealth Sustainably and Equitably*, World Resources Institute
- (7) Thrupp, L.A. (1998). Linking Biodiversity and Agriculture: Challenges and Opportunities for Sustainable Food Security. World Resources Institute.
- (8) for example, Centre for Applied Microbiology & Research, see <http://www.camr.org.uk>
- (9) <http://www.ukncc.co.uk/html/Information/Training%20Courses.htm>
- (10) <http://www.ukncc.co.uk/html/Information/project1.htm>
- (11) Poutanen S.M., Low, D.E., Henry, B., Finkelstein, S., Rose, D., Green, K., Tellier, R., Draker, R., Adachi, D., Ayers, M., Chan, A.K., Skowronski, D.M., Salit, I., Simor, A.E., Slutsky, A.S., Doyle, P.W., Krajden, M., Petric, M., Brunham, R.C., McGeer, A.J. (2003) Identification of severe acute respiratory syndrome in Canada. *N Engl J Med.* **348(20)**:1948-51.
- (12) Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YS, Khattri J, Asano JK, Barber SA, Chan SY, Cloutier A, Coughlin SM, Freeman D, Girn N, Griffith OL, Leach SR, Mayo M, McDonald H, Montgomery SB, Pandoh PK, Petrescu AS, Robertson AG, Schein JE, Siddiqui A, Smailus DE, Stott JM, Yang GS, Plummer F, Andonov A, Artsob H, Bastien N, Bernard K, Booth TF, Bowness D, Czub M, Drebot M, Fernando L, Flick R, Garbutt M, Gray M, Grolla A, Jones S, Feldmann H, Meyers A, Kabani A, Li Y, Normand S, Stroher U, Tipples GA, Tyler S, Vogrig R, Ward D, Watson B, Brunham RC, Krajden M, Petric M, Skowronski DM, Upton C, Roper RL.(2003) The

Genome sequence of the SARS-associated coronavirus. *Science*. **300(5624)**:1399-404.  
Epub 2003 May 01.

(13) Hurst, C. J. and Adcock, N. J. (2000) Relationship Between Humans and their Viruses, in *Viral Ecology*, ed. C. J. Hurst, Academic Press, San Diego

(14) Steinhauer DA, Skehel JJ. (2002) Genetics of Influenza Viruses, *Annu Rev Genet*. **36**:305-32

(15) Bushman, F. (2002), *Lateral DNA Transfer, Mechanisms and Consequences*, Cold Spring Harbor Press, Cold Spring Harbor, NY.

(16) Hall RA, Nisbet DJ, Pham KB, Pyke AT, Smith GA, Khromykh AA. (2003) DNA Vaccine Coding for the Full-Length Infectious Kunjin Virus RNA Protects Mice Against the New York Strain of West Nile Virus. *Proc Natl Acad Sci U S A*. [Epub ahead of print].

(17) Hart CA, Bennett M. (1999) Hantavirus Infections: Epidemiology and Pathogenesis. *Microbes Infect.*; **1(14)**:1229-37.

(18) Holmes EC. (2001) On the Origin and Evolution of the Human Immunodeficiency Virus (HIV). *Biol Rev Camb Philos Soc.*; **76(2)**:239-54.

(19) <http://www.wfcc.info/committee/endangered/home.html>

(20) Weldon, J., Ferguson, J., and Shindler, D. (1986) *Directory of Canadian Culture Collections 1986*, Minister of State for Science and Technology

(21) Fetch, T. (2003) Status of Microbial Genetic Resources and Culture Collections in Canada Workshop, Canadian Phytopathological Society Annual Meeting

(22) Task Force on the Status of Culture Collections in Canada, 1988, *Culture Collections in Canada*, Industry, Science and Technology Canada

(23) Beeler, J. A. *Human and Animal Viruses, in Maintaining Cultures for Biotechnology and Industry*, eds. Hunter-Cevera, J.C. and A. Belt, 1996, Academic Press, NY

(24) Joklik, W. K., Willett, H. P., Amos, D. B., Wilfert, C. M. (1992) *Zinsser Microbiology*, 20th Ed., Appleton & Lange, Norwalk

(25) Fields, B. N. (2001) *Field's Virology*, 3 ed, Lippincott Williams & Wilkins, NY

(26) Mahy, B. W. (1997) *A Dictionary of Virology*, 2nd Ed., Academic Press, San Diego

(27) Departments of the Army, the Navy and the Airforce (1983) NATO Handbook on the Medical Aspects of NCB Defensive Operations, FM 8-9, NAVMED P-5059, AFJMAN 44-151

(28) <http://www.bt.cdc.gov/agent/agentlist.asp>

(29) Hill, L. R., Kirsop, B. E. (1991) *Living Resources for Biotechnology: Bacteria*, Cambridge University Press, Cambridge

(30) <http://www.appliedbiosystems.com>

(31) <http://www.ukncc.co.uk/html/Information/project2.htm>

(32) Tanaka, Y., Yoh, M., Takeda, Y., Miwatami, T. (1979) Induction of mutation in *E. coli* by freeze-drying. *Appl. Environ. Microbiol.* **37**, 369-372

(33) World Data Centre for Microorganisms: <http://www.wdcm.org/>

(34) <http://www.wdcm.org/hpcc.html>

(35) <http://www.ucalgary.ca/~kesander/>

(36) <http://ictvdb.bio2.edu/index.htm>.

(37) <http://www.cabri.org>

(38) <http://panizzi.shef.ac.uk/msdn/>

(39) <http://www.wdcm.org/>

(40) <http://www.wdcm.org/hpcc.html>

(41) Stigler, L. (2003) Culture Collections in Canada: Perspectives and Problems, *Can. J. Phytopath.* In press

## Appendix I - BW Agent Lists

CDC BW Agent List

(see <http://www.bt.cdc.gov/agent/agentlist.asp>)

### Bacteria:

Bacillus anthracis (anthrax)  
 Brucella species (brucellosis)  
 Burkholderia mallei (glanders)  
 Burkholderia pseudomallei (melioidosis)  
 Chlamydia psittaci (psittacosis)  
 Cholera (Vibrio cholerae )  
 Clostridium botulinum toxin (botulism)  
 Clostridium perfringens (Epsilon toxin)  
 Coxiella burnetii (Q fever)  
 Escherichia coli O157:H7 (E. coli )  
 Francisella tularensis (tularemia)  
 Rickettsia prowazekii (typhus fever)  
 Salmonella species (salmonellosis)  
 Salmonella Typhi (typhoid fever)  
 Shigella (shigellosis)  
 Vibrio cholerae (cholera)  
 Yersinia estis (plague)

### Viruses:

arenaviruses [e.g., Lassa, Machupo]  
 Eastern Equine Encephalitis  
 filoviruses [e.g., Ebola, Marburg]  
 hantavirus  
 Nipah virus  
 Variola major (smallpox)  
 Venezuelan Equine Encephalitis  
 Western Equine Encephalitis

NATO Handbook on the Medical Aspects of NBC Defensive Operations  
 US Army Field Manual 8-9, Navy Medical Publication 5059, Air Force Joint  
 Manual 44-151  
 Annex A List

Note : species names in brackets are those used in this study

### Bacterial:

Anthrax (Bacillus anthracis)  
 Brucellosis (Brucella sp.)

Cholera (*Vibrio cholera*)  
 Melioidosis (*Burkholderia pseudomallei*)  
 Plague - pneumonic (*Yersinia pestis*)  
 Shigella (*Shigella* sp.)  
 Tularemia (*Francisella tularensis*)  
 Typhoid fever (*Salmonella typhi*)

**Rickettsial:**

Epidemic typhus (*Rickettsia prowazekii*)  
 Q fever (*Coxiella burnetii*)  
 Rocky Mountain spotted fever (*Rickettsia rickettsii*)  
 Scrub typhus (*Rickettsia tsutsugamushi*)

**Chlamydial:**

Psittacosis (*Chlamydia psittaci*)

**Fungal:**

Coccidioidomycosis (*Coccidioides immitis*)  
 Histoplasmosis (*Histoplasma capsulatum*)

**Viral**

Argentine hemorrhagic fever (*Junin virus*)  
 Bolivian hemorrhagic fever (*Machupo virus*)  
 Chikungunya fever (*Chikungunya virus*)  
 Crimean-Congo hemorrhagic fever (*Crimean-Congo hemorrhagic fever virus*)  
 Dengue fever (*Dengue virus*)  
 Ebola (*Cote d'Ivoire Ebola virus, Reston Ebola virus, Sudan Ebola virus, Zaire Ebola virus*)  
 Eastern equine encephalitis (*Eastern equine encephalitis virus*)  
 Influenza (*Influenza A, B and C virus*)  
 Korean hemorrhagic fever (*Hantaan*) (*Hantaan virus*)  
 Lassa (*Lassa virus*)  
 Omsk hemorrhagic fever (*Omsk hemorrhagic fever virus*)  
 Rift Valley fever (*Rift Valley fever virus*)  
 Russian spring-summer encephalitis (*Tick-borne encephalitis virus*)  
 Smallpox (*Variola virus*)  
 Venezuelan equine encephalitis (*Venezuelan equine encephalitis virus*)  
 Yellow fever (*Yellow Fever virus*)



**Toxins:**

Botulinum toxins (*Chlostrium botulinum*)

Clostridial perfringens toxins (*Clostridium perfringens*)

Mycotoxins of the trichothecene group (*Fusarium* sp.)

Palytoxin (coral species - outside survey)

Ricin (castor bean plant - outside survey)

Saxitoxin (dinoflagellates - outside survey)

Staphylococcal enterotoxins (*Staphylococcus aureus*)

Tetrodotoxin (pufferfish - outside survey

tside survey)

**Appendix II – Culture Collection Procedure References**

Hill, L. R., Kirsop, B. E. (1991) *Living Resources for Biotechnology: Bacteria*, Cambridge University Press, Cambridge

Hunter-Cevera, J. C. Belt, A. (1996) *Maintaining Cultures for Biotechnology and Industry*, Academic Press, NY

Kirsop, B.E. (1979) *The Stability of Industrial Organisms*, Commonwealth Mycological Institute, Kew, Surrey, England

Kirsop, B. E., Snell, J. J. S. (1984) *Maintenance of Microorganisms*, Academic Press, NY

Smith, D. Onions, A. H. S. (1983) *The Preservation and Maintenance of Living Fungi*, Commonwealth Agricultural Bureaux, England

**Appendix III - Web Collection Search-Engine Sample and Sample Output**

---

**CMCN Datasheet**

---

***Aspergillus Acanthosporus***

<b>Collection:</b>	CABI - Collection of Filamentous Fungi and Plant Pathogenic Bacteria
<b>Strain Number:</b>	164621
<b>Organism Type:</b>	Fungus
<b>Other Collections:</b>	NHL 2462; ATCC 22931; CBS 558.71
<b>Status:</b>	T of <i>Aspergillus acanthosporus</i> Udagawa & Takada
<b>Isolated From:</b>	soil
<b>Geographic Origin:</b>	Solomon Is.
<b>Identified By:</b>	A.H.S. Onions
<b>Form Of Supply:</b>	Freeze Dried
<b>Conditions For Growth:</b>	CZ, MCZ, 23

---



## Appendix IV – Database Item / Entry Outline

### Basic Filing / Search Information

-intended for public viewing

-items 1 to 8 are entered immediately upon the strain being received, as a ‘preliminary’ to further documentation

-items 9-13 are publicly available but entered later during the acquisition process

- 1) Accession Number - collection’s unique master-reference number
  - automatically assigned when a new record is created
- 2) Organism Kind - list of choices, likely limited to :
  - Eubacteria
  - Archaeobacteria
  - Virus
  - Fungi
  - Protozoa
  - Plant
  - Animal
  - the last three won’t likely be used in the initial sets
- 3) Taxonomic Position
  - A) Kingdom
  - B) Subkingdon
  - C) Phylum
  - D) Subphylum
  - E) Superclass
  - F) Class
  - G) Subclass
  - H) Superorder
  - I) Order
  - J) Suborder
  - K) Superfamily
  - L) Family
  - M) Subfamily
  - O) Supergenus
  - P) Genus
  - Q) Subgenus
  - R) Superspecies
  - S) Species
  - T) Subspecies
  - each a text box with a pull-down option of previously entered examples
  - note not each one is used or necessary except for Genus, and almost always species
  - Species has a special wildcard entry - “sp.” which indicates this is a species of the genus specified, but not determined which.
- 4) Strain ID - may be accession number of another culture collection, the name given by someone submitting the sample, eg. strain A31/2 clone 3.
  - lower than subspecies in hierarchy position

- no real pattern, no official international standardization.
- enter as a text block
- 5) Alternative / Obsolete / Common Names
  - may be several, very important to be searchable by end-users
  - typically will be a 'genus species' name - eg. *Enterobacter floges*
  - may occasionally be a 'common name', eg Spanish flu
- as old names become obsolete, will likely need to add to this list
- 6) Additional Strain Characteristics
  - text block, too variable to subdivide/predict
  - includes data such as resistances, metabolism, products produced, unique or unusual features of the strain
  - includes data such as where strain originates
  - for pathogens, likely include hosts
- 7) Strain Open Science Literature Description
  - text block
  - key journal articles / paper where organism and strain are described
  - alternatively a patent document
- 8) Health risk level
  - pull down menu for four basic threat levels (BL1-4)
  - additional text block for notes

Note - items below are added to the database later once determined

- 9) Standard growth conditions for the strain
  - text block describing growth conditions
- 10) Appearance information
  - A) colony or plaque description
  - B) organism microscopic appearance description
  - C) photo of colony/plaque
  - D) photo of microscopic appearance
  - items A, B are text blocks
- 11) Preservation protocol
  - text block
- 12) Storage Conditions
  - likely a limited number of options, a pull-down menu with possibility of typing new options
- 13) Kind of Preserved Sample
  - probably limited number of options, a pull-down menu with possibility of typing new options

### Acquisition Data

- data entered during initial processing of strain
- most will not be available to public

- 1) Date Acquired
- 2) Source of Strain
  - likely a pull down menu with ability to type in new sources
- 3) Form Acquired
  - likely a pull down menu with ability to type in new sources
- 4) Condition Acquired
  - check buttons - intact / damaged
  - additional text block to describe damage if necessary
- 5) Initial Growth Conditions
  - text block for describing the medium, temperature, atmosphere etc used to initially grow the culture
- 6) Source of Original Growth Protocol
  - text block for where-ever the original technique was found
- 7) Initial Growth Successful
  - was culture viable/was there obvious growth? Yes/No
  - if not, return to step 5 and have a new set of growth conditions
  - OR - terminate accession - organism is not-viable, no further data entered
- 8) Contamination
  - was culture obviously contaminated? Yes/No
  - if yes, describe contamination (text block)
  - if yes, describe technique(s) used to purify (text block)
  - unable to eliminate contamination? no further data entered
- 9) Is Organism Correctly ID'd?
  - description of how confirm organism ID, text block
- 10) Record standard growth conditions for organism at culture collection (note also public info)
- 11) Record Appearance information
  - A) colony or plaque description
  - B) organism microscopic appearance description
  - C) photo of colony/plaque
  - D) photo of microscopic appearance
  - items A, B are text blocks
  - note - also public info
- 12) nucleic acid info if obtained during acquisition
  - not yet determined what kind of information will be stored here, for bacteria/fungi will likely be a rRNA sequence obtained via PCR and sequencing. Currently more a place-holder, later fill in
  - for initial database use a text block

### Growth and Preservation Data

- data entered during process of growing and storing sample for long-term storage
- most data won't be available to the public

- 1) Source of Growth/Storage Protocol
  - text block for original technique
- 2) Conditions Used to Grow Culture for Storage
  - conditions used described in a text-block
- 3) Cell Count after Growth
  - number, cells / ml
- 4) Storage Conditions
  - likely a pull-down menu with ability to type new options
  - eg liquid nitrogen, liquid nitrogen vapor, lyophilized + -70, lyophilized and fridge etc
  - note - also available to public
- 5) Kind of Preserved Sample
  - eg stored in plastic cryo-tube, in glass lyophile
  - likely a pull-down menu with ability to type new options
  - note - also available to public
- 6) Storage Protocol
  - text block
  - note - also available to public
- 7) Cell Count after Preservation
  - number, cells/ml
- 8) Was preservation successful?
  - No If #7 is too much lower than #3, repeat procedure 2 onward
  - If Yes then :
    - Activate record for public access, strain is now available for request
- 9) Date Samples Preserved
- 10) Physical Location of Samples
  - A) Number of samples
  - B) volume per sample
  - C) where stored
    - freezer/vat number/name
    - box/hanger
    - vial position/number
  - Assign each vial a unique identity number/bar code?



### Viability Check Data

-data will be entered each time a strain is tested for continuing viability.

-data will not be available to the general public

- 1) Vial ID for sample recovered from storage then re-grown  
-ID number
- 2) Date of Recovery / Re-growth  
-date
- 3) Cell Count after Growth  
-number, cells / ml
- 4) Contamination  
-was culture obviously contaminated? Yes/No  
-if yes, describe contamination (text block)  
-if yes, describe technique(s) used to purify (text block)  
-unable to eliminate contamination? no further data entered
- 5) Does culture need to be re-grown?  
-if #3 is too low, or contamination has been found then yes, otherwise no  
-if no, when need to do next check (date)  
-if yes:

Repeat Growth and Preservation Steps 2 to 10 (listed below), likely have each default to the data entered in the Growth and Preservation dataset, but allow each to be edited.

- 6) Conditions Used to Grow Culture for Storage  
-conditions used described in a text-block
- 7) Cell Count after Growth  
-number, cells / ml
- 8) Storage Conditions  
-likely a pull-down menu with ability to type new options  
-eg liquid nitrogen, liquid nitrogen vapor, lyophilized + -70, lyophilized and fridge etc  
-note - also available to public
- 9) Kind of Preserved Sample  
-eg stored in plastic cryo-tube, in glass lyophile  
-likely a pull-down menu with ability to type new options  
-note - also available to public
- 10) Storage Protocol  
-text block  
-note - also available to public
- 11) Cell Count after Preservation  
-number, cells/ml
- 12) Was preservation successful?  
No If #11 is too much lower than #7, repeat procedure 2 onward  
If Yes then :  
Activate record for public access, strain is now available for request
- 13) Date Samples Preserved

14) Physical Location of Samples

- A) Number of samples
- B) volume per sample
- C) where stored
  - freezer/vat number/name
  - box/hanger
  - vial position/number

Assign each vial a unique identity number/bar code?

### Sample Dispatch

- data entered whenever a sample is requested and sent
- information will not be publicly available

- 1) Order Number / ID
  - a sequential number?
- 2) Individual Making Request
  - series of text boxes, including
    - A) Name
    - B) Address
    - C) Other Contact Info (eg email address, phone number)
- 3) Date of Request
  - date box
- 4) Sample Requested
  - identify using accession number (see public data)
- 5) If Sample Restricted, is Recipient Certified to Receive?
  - variable contents depending on kind of collection planned - will depend significantly on what kind of certification process has been developed. Initially plan for a text block for entering data
- 6) Test Sample to Verify Identity / Contamination
  - vial number retrieved to test
- 7) Growth Conditions Used
  - default is Acquisition Data point 5, may be edited
- 8) Number Surviving Organisms
  - alive or not
  - enter number (cells / ml)
- 9) Contamination
  - yes / no
  - if yes, describe contamination ( text block)
- 10) If sample is alive (step 8) and not contaminated (step 9) then sample is dispatched
  - if sample is not alive or contaminated
    - A) attempt to re-grow (items 6 to 14 on viability check procedure
    - B) if contaminated:
      - if yes, describe technique(s) used to purify (text block)
    - C) if A and B successful, dispatch sample
      - if A or B fail, cancel order, also go to Retire Strain listing
- 11) Shipping Data
  - A) date shipped
  - B) method shipped (pull-down menu?)
    - order tracking number / other postal identification
  - C) packaging method (pull-down menu of options?)
  - D) instructions / data for sample
    - this should be a record of the information which accompanied the requested strain, and would include:
      - items 1 to 4 of Sample Dispatch
      - the identity number of the vial dispatched

- all then current information in the Basic Filing / Search Information set
- the entire instruction set would be printed for the recipient, and also saved in a text form by the collection for later reference. Both the printed report and the recorded text version
- would be generated automatically

### Retire Sample

-data entered when a strain is no longer being kept within the collection, either as it has died or has been selectively removed (due to space limitations, duplication, etc)

-not publicly available

- 1) Accession Number (automatically entered)
- 2) Organism Name - Genus/species, automatically obtained from public / basic information)
- 3) Date Retired
- 4) Reason Retired  
-likely a text block
- 5) Remove Sample from Public List  
-activate a tag which removes from the collection's searchable library, but retain all other data for record-keeping needs

## **Appendix V - Culture Collection Training Sources**

### CBS - Centraalbureau voor Schimmelcultures

Training : mycology, identification and handling procedures  
<http://www.cbs.knaw.nl/courses/index.htm>

### DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

training : database, sample preparation / procedures, taxonomy  
<http://www.dsmz.de/lst/index.html>

### National Collection of Pathogenic Fungi

training : collection management, isolation, identification, characterisation, growth and preservation  
[http://www.ukncc.co.uk/html/members/Ncpf/Ncpf\\_info.htm](http://www.ukncc.co.uk/html/members/Ncpf/Ncpf_info.htm)

### National Collection of Pathogenic Viruses

<http://www.ukncc.co.uk/html/members/ncpv/ncpv.htm>

### National Collection of Type Cultures

training : collection management, isolation, identification, characterisation, growth and preservation  
[http://www.ukncc.co.uk/html/members/nctc/nctc\\_info.htm](http://www.ukncc.co.uk/html/members/nctc/nctc_info.htm)

### National Institute for Biological Standards and Control (NIBSC)

training : quality control, documentation (note - viral vaccine focus)  
<http://www.nibsc.ac.uk/gtn.html>

### University of Alberta Microfungus Collection

training : taxonomy, contamination, preservation, handling  
<http://www.devonian.ualberta.ca/uamh/training.htm#Individual%20training>

### WFCC/USFCC/CCMM/BCCM Training Course

training : all aspects of organization, management, operation and conservation of microbial collections

<http://wdec.nig.ac.jp/wfcc/terb.html>

<http://www.wfcc.info/activities.html>

Note - offered periodically by the World Federation of Culture Collections. Next session May 3-7, 2004.

## Appendix VI - Culture Collection Strain Holding / Staff

Collection Name	WDCM	Staff			V/B/F	# of Strains	Ratio of Strains to Staff		
		Res	Supp	Total			Res	Supp	Total
New Zealand Reference Culture Collection, Medical Section	457	1	0	1	B	4000	4000	-	4000
Belgian Coordinated Collections of Microorganisms	296	5	9	14	B	18000	3600	2000	1286
Belgium Bacterial Culture Collection	400				B	3500	-	-	-
Belgium BCCM/IHEM	642	18	12	30	F	13000	722	1083	433
Czech Animal Pathogens Institute	181	1	1	2	B	594	594	594	297
Czech Animal Pathogens Institute	181	3	1	4	V	328	109	328	82
Czech Collection of Fungi	182	3	3	6	F	1800	600	600	300
Czech Collection of Microorganisms	64	5	1.5	6.5	B	2400	480	1600	369
Czech Collection of Microorganisms	64	1	1.5	2.5	F	850	850	567	340
Czech National Collection of Type Culture	130	11	8.5	19.5	B	5500	500	647	282
Czech National Collection of Type Culture	130	17	8.5	25.5	V	650	38	76	25
Denmark IBTCulture Collection of Fungi	758	7	10	17	F	22000	3143	2200	1294
Estonia Tartu Fungal Collection	821	1	0	1	F	2400	2400	-	2400
France Collection Francaise des Bacteries Phytopathogenes	629	5	0	5	B	3900	780	-	780
France Collection Nationale de Cultures de Microorganismes	174	1	1	2	B	1725	1725	1725	863
France Collection Nationale de Cultures de Microorganismes	174	2	1	3	V	194	97	194	65
Germany Institute of Virology	429	1	1	2	V	120	120	120	60
Germany Medical Culture Collection Marburg	418	2	0	2	B	3000	1500	-	1500
Hungarian National Collection of Medical Bacteria	258	2	2	4	B	3350	1675	1675	838
Hungary Collection of Animal Viruses	427	1	0	1	V	36	36	-	36
Indian Type Culture Collection	430	3	2	5	F	2500	833	1250	500
Japan Collection of Microorganisms	567	2	1	3	B	6400	3200	6400	2133
Japan Collection of Microorganisms	567	1	1	2	F	1200	1200	1200	600
Korean Collection for Type Cultures	597	6	2.5	8.5	B	5000	833	2000	588
Korean Collection for Type Cultures	597	2	2.5	4.5	F	400	200	160	89
NCCB, the Netherlands Culture Collection of Bacteri	797	2	2	4	B	10000	5000	5000	2500
Netherlands Centraalbureau voor Schimmelcultures	133	10	19	29	F	40500	4050	2132	1397
New Zealand Reference Culture Collection, Medical Section	457	1	0	1	B	4000	4000	-	4000
Norway Medical Microbiological Laboratory	432			0	V	1900	-	-	-
Polish Collection of Microorganisms	106	2	0	2	B	2200	1100	-	1100
Sweden Culture Collection, University of Goteborg	32	3	2	5	B	40000	13333	20000	8000
UK National Collection of Pathogenic Fungi	184	2	1	3	F	1300	650	1300	433
UK National Collection of Pathogenic Viruses	814	3	1	4	V	180	60	180	45
UK National Collection of Type Cultures	154	6	4	10	B	5000	833	1250	500
US ATCC	1	16		16	B	17000	1063	-	1063
US ATCC	1	11		11	F	46000	4182	-	4182
US ATCC	1	6		6	V	2200	367	-	367

WDCM - World Data Centre for Microorganisms (<http://www.wdcm.org/>) culture collection number

Res - researchers / scientists

Supp - support staff / technicians

Total - both research and support staff

V/B/F - virus, bacteria or fungi

**Appendix VII - BL 3/4 + BL Agent Pathogen List**

BACTERIA	BW Agent		BL Level		Unusual Need			Preservation		Path. Strain:	
	list	NATC list	clinical testing	bulk prod.	Complex Media	Atmos	Parasitic Culture	Lyoph	Cryo	ATCC	NCI UKN
Bacillus anthracis	+	+	2	3						*	
Bordetella pertussus			2	3	+					21	
Brucella abortus	+	+	2	3	+	+				*	
Brucella canis	+	+	2	3	+	+				1	
Brucella melitensis	+	+	2	3	+	+				*	
Brucella suis	+	+	2	3	+	+				*	
Burkholderia pseudomallei	+	+	2	3						0	
Chlamydia pneumonia			2	3			+			*	
Chlamydia psittaci	+	+	2	3			+			*	
Chlamydia trachomatis			2	3			+			*	
Chlostridium botulinum	+	+	2	3		+				*	
Clostridium perfringens	+	+	2	3	+					58	
Coxiella burnetii	+	+	2	3			+			*	
Escherichia coli O157:H7	+	+	2	3						1	
Francisella tularensis	+	+	2	3						1	
Legionella sp.			2	3	+					37	
Legionella pseudophila			2	3	+					1	
Mycobacterium bovis			2	3	+					35	
Mycobacterium tuberculosis			2	3	+					46	
Neisseria gonorrhoeae			2	3	+					81	
Neisseria meningitidis			2	3	+					86	
Rickettsia akari			2	3						*	
Rickettsia australis			2	3			+			*	
Rickettsia conorii			2	3			+			*	
Rickettsia japonicum			2	3			+			*	
Rickettsia prowazekii		+	2	3			+			*	
Rickettsia rickettsii	+	+	2	3			+			*	
Rickettsia siberica			2	3			+			*	
Rickettsia tsutsugamushi		+	2	3			+			*	
Rickettsia typhi			2	3			+			*	









**Appendix VIII - Pathogenic Bacteria**

Genus	Species	Unusual Need		Biosafety Levels Over 2	Preservation		Path. Strains / Collectio			ATCC Species Total	ATCC Genus Total	UKNCC Specie: Total
		Complex Media	Atmos Parsitic Culture		Can't Culture	Lyoph	Cryo	Path. Species	ATCC UKNCC			
Actinomadura	madurae							6	8	0	6	92
Actinomyces	israelii							7	9	0		
Actinomyces	meyeri	+						2	2	1		
Actinomyces	naeslundii	+						8	1	0		
Actinomyces	odontolyticus	+						3	2	2		
Actinomyces	pyogenes	+						2	0	1		
Actinomyces	viscosus	+						7	2	0	29	285
Aeromonas	caviae							7	16	57		
Aeromonas	hydrophila							0	0	0		
Aeromonas	sorbia							0	0	0	7	115
Anaerococcus	prevotii							2	0	0		
Anaerococcus	tetradius							1	0	1	1	11
Arcanobacterium	haemolyticum							1	9	1	1	13
Bacillus	anthracis			2/3				*	8	*		
Bacillus	cereus							62	53	44		
Bacillus	subtilis							100	89	19	162	999
Bacteroides	distasonis							1	1	0		
Bacteroides	fragilis	+						21	7	1		
Bacteroides	ovatus	+						2	1	0		
Bacteroides	thetaiotaomicron	+						5	2	1		
Bacteroides	ureolyticus	+						6	8	5		
Bacteroides	vulgatus	+						3	2	1	38	144
Bartonella	bacilliformis							2	5	0	2	15
Bifidobacterium	dentium							6	3	3	6	82
Bordetella	bronchiseptica	+						15	18	41		
Bordetella	parapertussis	+						8	10	20		
Bordetella	pertussis	+						21	15	12	44	54
Borrelia	burgdorferi							6	0	0	6	18
Bruceella	abortus	+		2/3				*	*	*		
Bruceella	melitensis	+		2/3				*	56	*		
Bruceella	ovis	+		2/3				1	*	*		
Bruceella	suis	+		2/3				*	*	*	1	83
Burkholderia	pseudomallei			2/3				0	12	0	0	86
Calymatobacterium	granulomatis	+						0	0	0	0	0
Campylobacter	coli							0	0	0	0	0
Campylobacter	fetus	+						29	15	20		
								10	5	2		

Genus	Species	Complex Media		Unusual Need		Biosafety Levels Over 2	Preservation		Path. Species	Path Strains / Collectio		ATCC Species Total	ATCC Genus Total	UKNCC Specie Total
		Complex Media	Atmos	Parasitic Culture	Can't Culture		Lyoph	Cryo		ATCC	UKNCC			
Campylobacter	gracilis		+						1	1	1	130	187	55
Campylobacter	jejuni		+						1	87	34	40		
Campylobacter	laridis		+						1	3	0	0		
Capnocytophaga	gingivalis								1	1	2	4		
Capnocytophaga	ochracea								1	3	5	6		
Capnocytophaga	sputigena								1	1	1	5	14	8
Cardiobacterium	hominis								1	7	3	1	7	3
Chlamydia	pneumoniae			+		2/3			1	*	*	*	0	0
Chlamydia	psittaci			+		2/3			1	*	*	*	0	0
Chlamydia	trachomatis			+		2/3			1	*	*	*	0	0
Chromobacterium	violaceum								1	12	30	33	27	30
Chryseobacterium	indologenes								1	2	0	5		
Chryseobacterium	meningosepticum								1	8	0	15	19	0
Citrobacter	amalonaticus								1	3	3	1		
Citrobacter	freundii								1	7	29	6		
Citrobacter	koseri								1	11	0	2	21	32
Clostridium	botulinum			+		2/3			1	*	23	*		
Clostridium	difficile			+					1	17	18	0		
Clostridium	fallax			+					1	2	2	0		
Clostridium	histolyticum			+					1	7	5	0		
Clostridium	novyi			+					1	13	17	1		
Clostridium	perfringens			+					1	58	66	4		
Clostridium	septicum			+					1	8	17	0		
Clostridium	sordellii			+					1	2	4	1		
Clostridium	tetani			+					1	7	30	0	407	182
Corynebacterium	bovis								1	2	12	0		
Corynebacterium	diphtheriae	+							1	33	52	0		
Corynebacterium	minutissimum	+							1	4	4	0		
Corynebacterium	pseudodiphtheriticum	+							1	14	8	0		
Corynebacterium	pseudotuberculosis	+							1	8	8	1		
Corynebacterium	xerosis	+							1	2	11	1		95
Coxiella	burnetii			+		2/3			1	*	*	*	722	0
Dermatophilosis	congolensis								1	1	0	0	1	0
Eggerthella	lenta								1	2	1	0	2	1
Ehrlichia	sennetsu								1	*	*	*	0	0
Eikenella	corrodens								1	3	2	1	3	2
Enterobacter	amnigenus								1	4	2	11	174	2
Enterococcus	durans								1	9	13	40		

Genus	Species	Unusual Need		Biosafety Levels Over 2	Preservation		Path. Species	Path. Strains / Collectio			ATCC Species Total	ATCC Genus Total	UKNCC Specie Total
		Complex Media	Atmos Parsitic Culture		Can't Culture	Lyoph		Cryo	ATCC	NCTC UKNCC			
Enterococcus	faecalis						1	84	65	188	133	184	143
Enterococcus	faecium						1	40	65	215	51	53	13
Erysipelothrix	rhusiopathiae						1	51	13	0			
Escherichia	blattae						1	3	2	1			
Escherichia	coli						1	100	100	32			
Escherichia	fergusonii						1	5	1	1			
Escherichia	hermannii						1	5	1	1	118	900	105
Escherichia	vulneris						1	5	1	1	1	100	0
Eubacterium	nodatum						1	1	0	0	0	1	0
Faenia	rectivirgula						1	0	1	0	0	0	1
Fluoribacter	bozemanii						1	2	3	0	2	11	3
Francisella	tularensis			2/3			1	1	1	0	1	4	1
Fusobacterium	mortiferum						1	2	0	0			
Fusobacterium	necrophorum						1	3	4	0			
Fusobacterium	nucleatum						1	7	2	1	12	37	6
Gardnerella	vaginalis						1	4	3	5	4	4	3
Gemella	morbilorum						1	2	3	1	2	7	3
Haemophilus	actinomycetemcomitans						1	8	6	0			
Haemophilus	aegyptius						1	26	3	0			
Haemophilus	ducreyi						1	12	8	0			
Haemophilus	influenzae						1	63	28	5			
Haemophilus	parainfluenzae						1	9	3	0	118	179	48
Helicobacter	pylori						1	18	16	5	18	75	16
Iodobacter	fluvialis						1	1	4	3	1	3	4
Klebsiella	oxytoca						1	22	18	29			
Klebsiella	planticola						1	5	6	24			
Klebsiella	pneumoniae						1	100	61	81			
Klebsiella	terrigena						1	8	5	34	135	166	90
Leclercia	adecarboxylata						1	9	3	23	9	9	3
Legionella	biflexa			2/3			1	1	0	0			
Legionella	pneumophila			2/3			1	37	34	0	38	123	34
Leptospira	interrogans						1	20	0	0			
Leptospira	micdadei						1	2	0	0	0	39	0
Listeria	monocytogenes						1	50	24	10	50	82	24
Mobiluncus	curtisi						1	2	0	1			
Mobiluncus	mulleris						1	3	0	1	5	5	0
Mycobacterium	avium						1	39	6	0			
Mycobacterium	bovis			2/3			1	35	6	0			

Genus	Species	Complex Media	Unusual Need		Biosafety Levels Over 2	Preservation		Path. Species	Path Strains / Collection				ATCC Species Total	ATCC Genus Total	UKNCC Species Total
			Atmos	Parsitic Culture		Can't Culture	Lyoph		Cryo	ATCC	UKNCC	NCTC			
Mycobacterium	chelonae	+						1	0	0	0	0			
Mycobacterium	fortuitum	+						1	33	18	0	0			
Mycobacterium	haemophilum	+						1	5	1	0	0			
Mycobacterium	intracellulare	+						1	53	2	0	0			
Mycobacterium	kansasii	+						1	17	1	0	0			
Mycobacterium	leprae	+						1	*	*	*	*			
Mycobacterium	lepraemurium	+						1	*	*	*	*			
Mycobacterium	malmoense	+						1	1	2	0	0			
Mycobacterium	marinum	+						1	9	14	0	0			
Genus	Species								ATCC	UKNCC	NCTC	BCCM			
Mycobacterium	microti	+						1	3	3	0	0			
Mycobacterium	scrofulaceum	+						1	20	1	0	0			
Mycobacterium	simiae	+						1	3	0	0	0			
Mycobacterium	szulgai	+						1	5	3	0	0			
Mycobacterium	tuberculosis	+			2/3			1	46	6	0	0			
Mycobacterium	ulcerans	+						1	8	5	0	0			
Mycobacterium	xenopi	+						1	14	1	0	0			69
Mycoplasma	hominis							1	15	1	0	0			
Mycoplasma	pneumoniae							1	12	1	0	0			2
Myroides	odoratus							1	2	4	4	4			5
Neisseria	gonorrhoeae	+			2/3			1	81	22	0	0			
Neisseria	meningitidis	+			2/3			1	86	45	0	0			67
Nocardia	asteroides							1	21	44	1	1			
Nocardia	brasiliensis							1	9	7	0	0			
Nocardia	ottidiscaviarum							1	6	2	0	0			53
Pasteurella	multocida							1	46	37	2	2			37
Peptoniphilus	asaccharolyticus							1	3	0	0	0			0
Peptostreptococcus	anaerobius							1	2	1	1	1			1
Plesiomonas	sp.							1	4	6	1	1			8
Plesiomonas	shigelloides							1	4	6	6	12			12
Porphyromonas	asaccharolytica							1	3	0	1	1			
Porphyromonas	gingivalis							1	5	0	0	0			0
Prevotella	bivia							1	1	1	1	1			
Prevotella	buccae							1	5	0	0	0			
Prevotella	corporis							1	1	0	0	0			
Prevotella	denticola							1	3	0	0	0			
Prevotella	disiens							1	1	0	0	0			
Prevotella	intermedia							1	4	0	0	0			
Prevotella	loescheii							1	2	0	0	0			

Genus	Species	Complex Media	Unusual Need		Biosafety Levels Over 2	Preservation		Path. Strains / Collection			ATCC Species Total	ATCC Genus Total	UKNCC Specie Total	
			Atmos	Parsitic Culture		Lyoph	Cryo	ATCC	UKNCC	BCCM				
Prevotella	melaninogenica							1	4	6	0	23	44	7
Prevotella	oris		+					1	2	0	0	0	0	0
Propionibacterium	acnes		+					1	11	1	6	12	61	1
Propionibacterium	propionica		+					1	1	0	0	0	0	0
Proteus	mirabilis							1	71	30	8	0	0	0
Proteus	penneri							1	3	3	0	97	160	59
Proteus	vulgaris							1	23	26	6	0	0	0
Pseudomonas	aeruginosa							1	100	100	40	0	0	0
Pseudomonas	cepacia							1	20	0	80	0	0	0
Pseudomonas	mallei							1	0	0	0	120	1469	100
Rhodococcus	equi							1	22	10	2	22	187	10
Rickettsia	akari		+		2/3			1	*	*	*	*	*	*
Rickettsia	australis			+	2/3			1	*	*	*	0	0	0
Rickettsia	conorii			+	2/3			1	*	*	*	0	0	0
Rickettsia	japonicum			+	2/3			1	*	*	*	0	0	0
Rickettsia	prowazekii			+	2/3			1	*	*	*	0	0	0
Rickettsia	rickettsii			+	2/3			1	*	*	*	0	0	0
Rickettsia	sibirica			+	2/3			1	*	*	*	0	0	0
Rickettsia	tsutsugamushi			+	2/3			1	*	*	*	0	0	0
Rickettsia	typhi			+	2.3			1	*	*	*	0	0	0
Rochalimaea	quintana			+				1	1	0	0	1	4	0
Salmonella	choleraesuis							1	100	4	15	126	293	5
Salmonella	typhi				2/3			1	26	1	28	0	0	0
Serratia	ficaria							1	2	1	0	0	0	0
Serratia	fonticola							1	7	1	0	0	0	0
Serratia	grimesii							1	3	3	1	0	0	0
Serratia	liquefaciens							1	8	4	2	0	0	0
Serratia	marcescens							1	76	53	7	0	0	0
Serratia	odorifera							1	4	1	0	0	0	0
Serratia	plymuthica							1	8	8	7	122	139	79
Serratia	rubidaea							1	14	8	1	0	0	0
Shigella	boydii							1	24	40	0	0	0	0
Shigella	dysenteriae							1	36	38	0	0	0	0
Shigella	flexneri							1	26	45	1	93	113	136
Shigella	sonnei							1	7	13	1	0	0	0
Sphingobacterium	multivorum							1	3	7	6	3	19	7
Spirillum	minus			+				1	0	0	0	0	65	0
Staphylococcus	aureus							1	100	100	38	0	0	0



Genus	Species	Complex Media		Unusual Need		Biosafety Levels Over 2	Preservation		Path. Species	Path Strains / Collection				ATCC Genus Total	UKNCC Specie: Total
		Complex Media	Atmos Parsitic Culture	Can't Culture	Lyoph		Cryo	ATCC		UKNCC	BCCM				
Staphylococcus	epidermidis								1	26	18	3	132	415	132
Staphylococcus	saprophyticus								1	6	14	1	1	62	14
Stenotrophomonas	maltophilia								1	54	14	100	54	8	8
Streptobacillus	moniliformis								1	8	8	0	0	8	0
Streptococcus	agalactiae								1	27	85	28	28	8	8
Streptococcus	anginosus								1	1	7	5	5	8	0
Streptococcus	constellatus								1	2	1	10	10	8	0
Streptococcus	dysgalactiae								1	12	19	74	74	8	0
Streptococcus	equisimilis								1	11	12	9	9	8	0
Streptococcus	intermedius								1	3	1	4	4	8	0
Streptococcus	milleri								1	0	7	0	0	8	0
Streptococcus	mitis								1	3	3	10	10	8	0
Streptococcus	mutans								1	16	9	4	4	8	0
Streptococcus	pneumoniae								1	100	35	4	4	8	0
Streptococcus	pyogenes								1	100	97	9	9	8	0
Genus	Species	Complex Media	Atmos Parsitic Culture	Can't Culture	Unusual Need	Biosafety Levels Over 2	Lyoph	Cryo	Path. Species	ATCC	UKNCC	BCCM	ATCC Species Total	ATCC Genus Total	UKNCC Specie: Total
Streptococcus	saiivarius								1	8	19	12	12	8	0
Streptococcus	sanguis								1	1	0	0	0	8	0
Streptococcus	zoepidemicus								1	12	0	0	0	685	295
Thermoactinomyces	vulgaris								1	7	5	0	7	20	5
Treponema	carateum			+					1	*	*	*	*	0	0
Treponema	pallidum			+					1	*	*	*	*	20	25
Treponema	pertenue			+					1	20	2	0	20	25	2
Ureaplasma	urealyticum								1	3	7	0	3	13	7
Veillonella	parvula		+						1	9	13	9	9	13	7
Vibrio	alginolyticus								1	24	48	*	*	20	0
Vibrio	cholera								1	1	0	1	1	25	2
Vibrio	cincinnatiensis								1	5	0	2	2	13	7
Vibrio	damsela								1	4	3	2	2	13	7
Vibrio	fluvialis								1	4	3	2	2	13	7
Vibrio	furnissii								1	5	4	6	6	13	7
Vibrio	hollisae								1	2	4	1	1	13	7
Vibrio	meschnikovii								1	0	0	0	0	13	7
Vibrio	mimicus								1	4	1	1	1	13	7
Vibrio	parahaemolyticus								1	18	43	10	10	328	123
Vibrio	vulnificus								1	16	7	4	4	328	123
Yersinia	enterocolitica								1	30	13	0	0	64	50
Yersinia	pestis					2/3			1	*	12	*	*	64	50
Yersinia	pseudotuberculosis								1	19	25	1	1	64	50

Totals:	45	52	17	4	29	226	3263	2531	1655	3231	11618	2531
---------	----	----	----	---	----	-----	------	------	------	------	-------	------

asterisk in strain counts indicates specimen present, but not made visible to public.

Data altered due to high species counts

Bacillus subtilis	1	197	89	19
Escherichia coli	1	720	735	32
Pseudomonas aeruginosa	1	333	219	40
Salmonella choleraesuis	1	266	4	15
Staphylococcus aureus	1	185	217	38
Streptococcus pneumoniae	1	118	35	4
Streptococcus pyogenes	1	144	97	9

Appendix IX- Pathogenic Viruses

Family	Genus	Species	Virus Abbreviation	Nucl. Acid	Human Only	Zoo.	Can't Culture	Biosafety Levels Over 2	Preservation Lyoph	Path. Species	ATCC	NCPV UKNCC	ATCC Specie Total
Adenoviridae	Mastadenovirus	Adenovirus	HAdV	DNA	+					1	78	71	7
Arenaviridae	Arenavirus	Guararito virus	GTOV	RNA	+			4		1	0	0	0
Arenaviridae	Arenavirus	Junin virus	JUNV	RNA	+			3		1	0	0	0
Arenaviridae	Arenavirus	Lassa virus	LASV	RNA	+			4		1	0	DNA	0
Arenaviridae	Arenavirus	Lymphocytic choriomeningitis virus	LCMV	RNA	+			3		1	2	7	7
Arenaviridae	Arenavirus	Machupo virus	MACV	RNA	+			4		1	0	0	0
Arenaviridae	Arenavirus	Sabia virus	SABV	RNA	+			4		1	0	0	0
Astroviridae	Mamastrovirus	Human astrovirus	HAsTV	RNA	+					1	0	7	7
Bunyaviridae	Hantavirus	Andes virus	ANDV	RNA	+			3		1	0	0	0
Bunyaviridae	Hantavirus	Bayou virus	BAYV	RNA	+			3		1	0	0	0
Bunyaviridae	Hantavirus	Black Creek Canal virus	BCCV	RNA	+			3/4		1	0	0	0
Bunyaviridae	Hantavirus	Dobrava-Belgrade virus	DOBV	RNA	+			3		1	0	3	3
Bunyaviridae	Hantavirus	Hantaan virus	HTNV	RNA	+			3		1	1	2	2
Bunyaviridae	Hantavirus	New York virus	NYV	RNA	+			3		1	0	0	0
Bunyaviridae	Hantavirus	Puumala virus	PUUV	RNA	+			3		1	1	7	7
Bunyaviridae	Hantavirus	Seoul virus	SEOV	RNA	+			3		1	0	2	2
Bunyaviridae	Hantavirus	Sin Nombre virus	SNV	RNA	+			3/4		1	0	2	2
Bunyaviridae	Hantavirus	Thailand virus	THAIV	RNA	+			3		1	0	0	0
Bunyaviridae	Nairovirus	Crimean-Congo hemorrhagic fever virus	C-CHFV	RNA	+			4		1	0	2	2
Bunyaviridae	Nairovirus	Dugbe virus	DUGV	RNA	+			3		1	0	0	0
Bunyaviridae	Nairovirus	Nairobi sheep disease virus	NSDV	RNA	+					1	0	0	0
Bunyaviridae	Orthobunyavirus	Bunyamwera virus	BUNV	RNA	+					1	11	0	0
Bunyaviridae	Orthobunyavirus	Bwamba virus	BWAV	RNA	+					1	4	0	0
Bunyaviridae	Orthobunyavirus	California encephalitis virus	CEV	RNA	+					1	11	2	2
Bunyaviridae	Orthobunyavirus	Carapuru virus	CARV	RNA	+					1	0	0	0
Bunyaviridae	Orthobunyavirus	Catu virus	CATUV	RNA	+					1	2	0	0
Bunyaviridae	Orthobunyavirus	Guama virus	GMAV	RNA	+					1	4	0	0
Bunyaviridae	Orthobunyavirus	Guaroa virus	GROV	RNA	+					1	1	0	0
Bunyaviridae	Orthobunyavirus	Madrid virus	MADV	RNA	+					1	1	0	0
Bunyaviridae	Orthobunyavirus	Marituba virus	MTBV	RNA	+					1	7	0	0
Bunyaviridae	Orthobunyavirus	Nyando virus	NDV	RNA	+					1	2	0	0
Bunyaviridae	Orthobunyavirus	Oriboca virus	ORIV	RNA	+					1	3	0	0
Bunyaviridae	Orthobunyavirus	Oropouche virus	OROV	RNA	+			3		1	3	0	0
Bunyaviridae	Orthobunyavirus	Shuni virus	SHUV	RNA	+					1	0	0	0
Bunyaviridae	Orthobunyavirus	Simbu virus	SIMV	RNA	+					1	1	0	0

Family	Genus	Species	Virus Abbreviation	Nucl. Acid	Human Only	Zoo.	Can't Culture	Biosafety Levels Over 2	Preservation Lyoph	Path. Species	ATCC	NCPV UKNCC	ATCC Specie Total
Bunyaviridae	Orthobunyavirus	Wyeomyia virus	WYOV	RNA						1	3	0	5
Bunyaviridae	Phlebovirus	Candiru virus	CDUV	RNA	+	+				1	1	0	
Bunyaviridae	Phlebovirus	Punta Toro virus	PTV	RNA	+	+				1	1	0	
Bunyaviridae	Phlebovirus	Rift Valley fever virus	RVFFV	RNA	+	+		3/4		1	0	0	
Bunyaviridae	Phlebovirus	Sandfly fever Naples virus	SFNV	RNA	+	+				1	2	4	
Caliciviridae	Norovirus	Norwalk virus	NV	RNA	+					1	0	0	
Coronaviridae	Coronavirus	Human coronavirus HCoV-229E	HCoV-229E	RNA	+					1	1	0	
Coronaviridae	Coronavirus	Human coronavirus HCV-OC43	HCoV-OC43	RNA	+					1	1	0	
Coronaviridae	Coronavirus	Sudden acute respiratory syndrome vi SARS		RNA	+					1	0	0	
Coronaviridae	Deltavirus	Hepatitis delta virus	HDV	RNA	+					1	2	0	
Coronaviridae	Torovirus	Human torovirus	HuTV	RNA	+					1	0	0	
Filoviridae	Ebola-like viruses	Cote d'Ivoire Ebola virus	CIEBOV	RNA		+	4			1	0	0	
Filoviridae	Ebola-like viruses	Reston Ebola virus	REBOV	RNA		+	4			1	0	0	
Filoviridae	Ebola-like viruses	Sudan Ebola virus	SEBOV	RNA		+	4			1	0	0	
Filoviridae	Ebola-like viruses	Zaire Ebola virus	ZEBOV	RNA		+	4			1	0	0	
Filoviridae	Marburg-like viruses	Marburg virus	MARV	RNA		+	4			1	0	0	
Flaviviridae	Flavivirus	Apoi virus	APOIV	RNA		+				1	0	0	
Flaviviridae	Flavivirus	Dengue virus	DENV	RNA		+				1	10	24	
Flaviviridae	Flavivirus	Ilheus virus	ILEV	RNA		+				1	2	0	
Flaviviridae	Flavivirus	Japanese encephalitis virus	JEV	RNA		+	3			1	0	4	
Flaviviridae	Flavivirus	Kokobera virus	KOKV	RNA		+				1	1	0	
Flaviviridae	Flavivirus	Kyasanur Forest virus	KFDV	RNA		+	4			1	0	0	
Flaviviridae	Flavivirus	Langat virus	LGTV	RNA		+				1	0	0	
Flaviviridae	Flavivirus	Louping ill virus	LIV	RNA		+	3			1	0	0	
Flaviviridae	Flavivirus	Murray valley encephalitis virus	MVEV	RNA		+	3			1	1	6	
Flaviviridae	Flavivirus	Omsk hemorrhagic fever virus	OHFV	RNA		+	4			1	0	0	
Flaviviridae	Flavivirus	Powassan virus	POWV	RNA		+				1	0	0	
Flaviviridae	Flavivirus	Sepik virus	SEPV	RNA		+	3			1	1	0	
Flaviviridae	Flavivirus	St. Louis encephalitis virus	SLEV	RNA		+	3			1	4	2	
Flaviviridae	Flavivirus	Tick-borne encephalitis virus	TBEV	RNA		+	4			1	0	6	
Flaviviridae	Flavivirus	Wesselbron virus	WESSV	RNA		+	3			1	9	0	
Flaviviridae	Flavivirus	West Nile virus	WNV	RNA		+	3			1	4	15	
Flaviviridae	Flavivirus	Yellow Fever virus	YFV	RNA		+	3			1	0	4	
Flaviviridae	Flavivirus	Zika virus	ZIKAV	RNA		+				1	1	0	3
Flaviviridae	Hepacivirus	Hepatitis C virus	HCV	RNA	+					1	0	0	
Flaviviridae	Hepacivirus	Hepatitis G virus	HGV-1	RNA	+					1	0	0	
Hepadnaviridae	Orthohepadnavirus	Hepatitis B	HBV	DNA	+					1	8	0	

Family	Genus	Species	Virus Abbreviation	Nucl. Acid	Human Zoo. Only	Can't Culture	Biosafety Levels Over 2	Preservation Lyoph	Preservation Cryo	Path. Species	ATCC	UKNCV	ATCC Specie Total
Herpesviridae	Cytomegalovirus	Human herpesvirus 5	HHV-5	DNA	+					1	4	8	
Herpesviridae	Lymphocryptovirus	Human herpesvirus 4	HHV-4	DNA	+					1	3	1	
Herpesviridae	Rhadinovirus	Human herpesvirus 8	HHV-8	DNA	+					1	0	0	
Herpesviridae	Roseolovirus	Human herpesvirus 6	HHV-6	DNA	+					1	1	4	
Herpesviridae	Roseolovirus	Human herpesvirus 6B	HHV-6B	DNA	+					1	1	0	
Herpesviridae	Roseolovirus	Human herpesvirus 7	HHV-7	DNA	+					1	0	0	
Herpesviridae	Simplexvirus	Cercopithecine herpesvirus 1	HVB	DNA	+		3			1	0	0	
Herpesviridae	Simplexvirus	Human herpesvirus 1	HHV-1	DNA	+					1	21	5	
Herpesviridae	Simplexvirus	Human herpesvirus 2	HHV-2	DNA	+					1	2	0	
Herpesviridae	Varicellovirus	Human herpesvirus 3	HHV-3	DNA	+					1	1	3	
Family	Genus	Species	Virus Abbreviation	Nucl. Acid	Human Zoo. Only	Can't Culture	Biosafety Levels Over 2	Preservation Lyoph	Preservation Cryo	Path. Species	ATCC	UKNCV	ATCC Specie Total
Orthomyxoviridae	Influenzavirus A	Influenza A virus	FLUAV	RNA	+					1	5	-	
Orthomyxoviridae	Influenzavirus B	Influenza B virus	FLUBV	RNA	+					1	8	-	
Orthomyxoviridae	Influenzavirus C	Influenza C virus	FLUCV	RNA	+					1	0	6	
Orthomyxoviridae	Thogotovirus	Thogoto virus	THOV	RNA	+					1	1	0	
Papovaviridae	Papillomavirus	Human papillomavirus	HPV	DNA	+					1	12	0	1
Paramyxoviridae	Heniparvirus	Nipahvirus	MeV	RNA	+					1	0	0	
Paramyxoviridae	Morbillivirus	Measles virus	MeV	RNA	+					1	1	20	
Paramyxoviridae	Pneumovirus	Human respiratory syncytial virus	HRSV	RNA	+					1	6	27	
Paramyxoviridae	Respirovirus	Human parainfluenza virus 1	HPIV-1	RNA	+					1	1	-	
Paramyxoviridae	Respirovirus	Human parainfluenza virus 3	HPIV-3	RNA	+					1	1	-	
Paramyxoviridae	Respirovirus	Human parainfluenza virus 2	HPIV-2	RNA	+					1	1	-	
Paramyxoviridae	Rubulavirus	Human parainfluenza virus 4	HPIV-4	RNA	+					1	2	4	
Paramyxoviridae	Rubulavirus	Mumps virus	MuV	RNA	+					1	3	0	
Parvoviridae	Dependovirus	Adeno-associated virus 1	AAV-1	DNA	+					1	1	0	
Parvoviridae	Dependovirus	Adeno-associated virus 2	AAV-2	DNA	+					1	1	0	
Parvoviridae	Dependovirus	Adeno-associated virus 3	AAV-3	DNA	+					1	1	0	
Parvoviridae	Dependovirus	Adeno-associated virus 4	AAV-4	DNA	+					1	1	0	
Parvoviridae	Dependovirus	Adeno-associated virus 5	AAV-5	DNA	+					1	0	0	
Parvoviridae	Dependovirus	Adeno-associated virus 6	AAV-6	DNA	+					1	0	0	
Parvoviridae	Parvovirus	LUIII virus	LUIII	DNA	+					1	1	0	
Picornaviridae	Cardiovirus	Encephalomyocarditis virus	EMCV	RNA	+					1	1	0	
Picornaviridae	Cardiovirus	Theilovirus	ThV	RNA	+					1	0	0	
Picornaviridae	Enterovirus	Human enterovirus A	HEV-A	RNA	+					1	-	-	
Picornaviridae	Enterovirus	Human enterovirus B	HEV-B	RNA	+					1	-	-	
Picornaviridae	Enterovirus	Human enterovirus C	HEV-C	RNA	+					1	-	-	
Picornaviridae	Enterovirus	Human enterovirus D	HEV-D	RNA	+					1	-	-	
Picornaviridae	Enterovirus	Human enterovirus E	HEV-E	RNA	+					1	4	25	

Family	Genus	Species	Virus Abbreviation	Nucl. Acid	Human Only	Human Zoo.	Can't Culture	Biosafety Levels Over 2	Preservation Lyoph	Path. Species	ATCC	UKNCC	NCPV UKNCC	ATCC Specie Total
Picornaviridae	Enterovirus	Poliovirus	PV	RNA	+						1	13	9	1
Picornaviridae	Hepatovirus	Hepatitis A virus	HAV	RNA	+						1	10	0	1
Picornaviridae	Kobuvirus	Aichi virus	AIV	RNA	+						1	0	0	0
Picornaviridae	Parechovirus	Human parechovirus	HPeV	RNA	+						1	1	0	0
Picornaviridae	Rhinovirus	Human rhinovirus A	HRV-A	RNA	+						1	-	0	0
Picornaviridae	Rhinovirus	Human rhinovirus B	HRV-B	RNA	+						1	104	107	10
Polyomaviridae	Polyomavirus	BK polyomavirus	BKPYV	DNA	+			3			1	0	0	0
Polyomaviridae	Polyomavirus	JC polyomavirus	JCPyV	DNA	+			3			1	3	0	0
Poxviridae	Molluscipoxvirus	Molluscum contagiosum virus	MOVC	DNA	+						1	0	0	0
Poxviridae	Orthopoxvirus	Buffalopox virus	BPXV	DNA	+	+					1	0	0	0
Poxviridae	Orthopoxvirus	Cowpox virus	CPXV	DNA	+	+					1	1	0	0
Poxviridae	Orthopoxvirus	Ectromelia virus	ECTV	DNA	+	+					1	1	0	0
Poxviridae	Orthopoxvirus	Vaccinia virus	VACV	DNA	+	+					1	15	6	6
Poxviridae	Orthopoxvirus	Variola virus	VARV	DNA	+	+					1	0	0	1
Poxviridae	Parapoxvirus	Bovine papular stomatitis virus	BPSV	DNA	+	+					1	1	0	0
Family	Genus	Species	Virus Abbreviation	Nucl. Acid	Human Only	Human Zoo.	Can't Culture	Biosafety Levels Over 2	Preservation Lyoph	Path. Species	ATCC	UKNCC	NCPV UKNCC	ATCC Specie Total
Poxviridae	Parapoxvirus	Orf virus	ORFV	DNA		+					1	1	0	0
Poxviridae	Parapoxvirus	Pseudocowpox virus	PCPV	DNA		+					1	1	0	0
Poxviridae	Yatapoxvirus	Tanapox virus	TANV	DNA		+					1	0	0	0
Poxviridae	Yatapoxvirus	Yaba monkey tumor virus	YMTV	DNA		+					1	1	0	0
Reoviridae	Coltivirus	Banna virus	BAV	RNA		+					1	0	0	0
Reoviridae	Coltivirus	Colorado tick fever virus	CTFV	RNA		+					1	0	0	0
Reoviridae	Coltivirus	Eyach virus	EYAV	RNA		+					1	0	0	0
Reoviridae	Orthoreovirus	Mammalian orthoreovirus	MRV	RNA		+					1	0	2	0
Reoviridae	Rotavirus	Rotavirus A	RV-A	RNA		+					1	-	0	0
Reoviridae	Rotavirus	Rotavirus B	RV-B	RNA		+					1	9	0	0
Retroviridae	Deltaretrovirus	Human T-lymphotropic virus 1	HTLV-1	RNA		+		2/3			1	0	0	0
Retroviridae	Deltaretrovirus	Human T-lymphotropic virus 2	HTLV-2	RNA		+		2/3			1	0	0	0
Retroviridae	Lentivirus	Human immunodeficiency virus 1	HIV-1	RNA		+		2/3			1	0	0	0
Retroviridae	Lentivirus	Human immunodeficiency virus 2	HIV-2	RNA		+		2/3			1	0	0	0
Retroviridae	Spumavirus	Chimpanzee foamy virus human isolat	CFV	RNA		+		2/3			1	0	0	0
Rhabdoviridae	Lyssavirus	Duvenhage virus	DUVV	RNA		+					1	0	0	0
Rhabdoviridae	Lyssavirus	Mokolo virus	MOKV	RNA		+					1	0	0	0
Rhabdoviridae	Lyssavirus	Rabies virus	RABV	RNA		+		2/3			1	5	0	0
Rhabdoviridae	Vesiculovirus	Chandipura virus	CHPV	RNA		+					1	0	0	0
Rhabdoviridae	Vesiculovirus	Piry virus	PIRYV	RNA		+		3			1	1	0	0
Rhabdoviridae	Vesiculovirus	Vesicular stomatitis Alagoas virus	VSAV	RNA		+		3			1	0	0	0
Rhabdoviridae	Vesiculovirus	Vesicular stomatitis Indiana virus	VSIV	RNA		+		3			1	5	-	-



## Appendix X Pathogenic Fungi

Genus	Species	Unusual Need		Path. Species	Preservation		Biosafety		Path Strains / Collection			ATCC		UKNCC		CE	
		Complex Media	Can't Culture		Lyoph	Cryo	Levels Over 2	ATCC	NCTC	UAMH	Species Total	Genus Total	Species Total	Genus Total	Species Total	Genus Total	Species Total
Absidia	corymbifera			1	v good	v good			29	28	23	17	29	99	28	28	
Acremonium	falciforme			1	v good	v good			3	0	3	0	3	249	0	144	
Alternaria	alternata			1	v good	v good			80	30	22	19					
Alternaria	chlamydospora			1	v good	v good			1	3	2	0	81	298	33	192	
Arthroderma	fulvum			1	v good	v good			6	0	8	7					
Arthroderma	grubyi			1	v good	v good			5	0	0	0	11	45	0	14	
Aspergillus	flavus			1	v good	v good			100	100	33	15					
Aspergillus	fumigatus			1	v good	v good			100	100	100	32					
Aspergillus	glaucus			1	v good	v good			1	5	0	0					
Aspergillus	nidulans			1	v good	v good			100	74	56	0					
Aspergillus	niger			1	v good	v good			100	100	100	14					
Aspergillus	terreus			1	v good	v good			56	44	26	16	457	1584	423	1444	3
Aureobasidium	pullulans			1	v. good?	v. good?			53	22	103	17	53	63	22	22	1
Bipolaris	hawaiiensis			1	v good	v good			16	4	8	1					
Bipolaris	spicifera			1	v good	v good			5	0	12	0	21	267	4	24	
Blastomyces	dermatitidis			1	v good	v good			39	9	0	0	39	54	9	40	
Blastoschizomyces	capitatus			1	v good	v good			15	0	0	0	15	16	0	0	
Candida	albicans			1	v good	v good			100	100	-	13					
Candida	glabrata			1	v good	v good			27	32	-	13					
Candida	guilliermondii			1	v good	v good			34	15	-	2					
Candida	kefyi			1	v good	v good			39	22	-	1					
Candida	krusei			1	v good	v good			26	36	-	5					
Candida	lusitaniae			1	v good	v good			45	64	-	0					
Candida	parapsilosis			1	v good	v good			45	23	-	3					
Candida	rugosa			1	v good	v good			7	5	-	0					
Candida	tropicalis			1	v good	v good			78	37	-	9	401	4921	334	540	-
Cladophialophora	bantiana			1	v good	v good			24	24	39	11	24	41	24	31	
Cladosporium	carrionii			1	v good	v good			9	0	27	0					
Cladosporium	devriesii			1	v good	v good			1	0	1	0	10	234	0	91	
Coccidioides	immitis			1	v good	v good	2/3		0	12	9	0	0	0	12	12	
Cochliobolus	spicifera			1	v good	v good			3	9	0	0	3	261	9	161	
Conidiobolus	coronata			1	fail	good			85	5	6	0	85	85	28	0	
Cryptococcus	albidus			1	good	v good			47	24	0	6					
Cryptococcus	gastricus			1	good	v good			3	1	0	0					



Genus	Species	Complex Media	Unusual Need		Biosafety Levels Over 2	Preservation		Path. Species	Path Strains / Collection			ATCC Species Total	ATCC Genus Total	UKNCC Species Total	UKNCC Genus Total	CE Sper Tot	
			Atmos	Parasitic Culture		Lyoph	Cryo		ATCC	UKNCC	CBS						UAMH
			Can't	+		good	v good		1	27	11						0
Cryptococcus	laurentii					good	v good	1	27	11	0	0	193	427	141	407	
Cryptococcus	luteolus					good	v good	1	6	2	0	0					
Cryptococcus	neoformans					good	v good	1	100	100	0	23					
Cryptococcus	terreus					good	v good	1	4	3	0	0					
Cryptococcus	uniguttulatus					good	v good	1	6	0	0	1					
Curvularia	geniculata					v good	v good	1	2	0	11	0					
Curvularia	lunata					v good	v good	1	34	15	18	6					
Epidermophyton	floccosum					v good	v good	1	16	17	11	8					
Exophiala	dermatitidis					v good	v good	1	27	76	53	31					
Exophiala	jeanselmei					v good	v good	1	28	56	63	65					
Exophiala	moniliae					v good	v good	1	3	1	7	7					
Exophiala	spinifera					v good	v good	1	13	5	25	10					
Exserohilum	rostratum					v. good?	v. good?	1	13	1	1	0					
Fonsecaea	pedrosoi					v good	v good	1	16	24	27	5					
Fusarium	solani					v good	v good	1	71	77	100	10					
Geotrichum	candidum					v good	v good	1	73	23	58	64					
Histoplasma	capsulatum					v good	v good	1	62	25	26	3					
Hormonema	dematoides				2/3	v. good?	v. good?	1	5	0	0	8					
Leptosphaeria	senegalensis					v good	v good	1	5	2	4	0					
Lacazia	loboi					v good	v good	1	0	0	0	0					
Madurella	grisea					v good	v good	1	6	16	2	3					
Madurella	mycetomi					v good	v good	1	11	23	0	0					
Malassezia	furfur					good	v good	1	18	15	0	7					
Malassezia	ovale					good	v good	1	5	0	0	0					
Microsporium	audouinii					v good	v good	1	23	9	23	5					
Microsporium	canis					v good	v good	1	40	39	0	20					
Microsporium	cookei					v good	v good	1	8	10	0	0					
Microsporium	distortum					v good	v good	1	3	13	0	8					
Microsporium	ferrugineum					v good	v good	1	3	1	8	9					
Microsporium	gallinae					v good	v good	1	5	4	0	0					
Microsporium	gypseum					v good	v good	1	43	23	16	27					
Microsporium	nanum					v good	v good	1	10	4	0	0					
Microsporium	persicolor					v good	v good	1	3	18	0	0					
Microsporium	rademosum					v good	v good	1	0	0	0	0					
Paracoccidioides	brasiliensis					v good	v good	1	27	3	10	1					
Phaeoannellomyces	werneckii					v. good?	v. good?	1	0	10	21	0					



## Data altered due to high species counts

Aspergillus flavus	1	176	130	33	15				
Aspergillus fumigatus	1	100	117	300	32				
Aspergillus nidulans	1	232	74	56	0				
Aspergillus niger	1	129	100	137	14				
Aureobasidium pullulans	1	53	22	103	17	53	63	22	22
Candida albicans	1	218	141	-	13				
Cryptococcus neoformans	1	135	353	0	23				
Fusarium solani	1	71	77	155	10	71	1289	77	557
Trichophyton mentagrophytes	1	65	42	29	170				
Yarrowia lipolytica	1	113	12	0	0	113	115	12	12

### Appendix XI Sample Equipment and Suppliers

Equipment	Manufacturer / Kind	Price Source	Price
Freeze Drying Equipment	Edwards Super Modulyo 12K	lab equip online	\$12,000
	Virtis Console Shelf Lyophilizer	lab equip online	\$12,000
Fridges	Manifold lyophilizer	lab equip online	\$6,000
	Benchtop freedryer 6L manifold	Cole-Parmer	\$13,000
Freezers	Console freezedryer 6L manifold/shelf	Cole-Parmer	\$14,000
	Stand-alone	Cole-Parmer	\$8,000
Freezers	Walk-in (8 x 8 x 7 ft)	various restaurant suppliers	\$5,000
	-30 chest freezer 22 cubic feet	Biocold Scientific	\$3,000
	-30 chest freezer 14 cubic feet	Biocold Scientific	\$2,200
	-30 chest freezer 5 cubic feet	Biocold Scientific	\$1,500
	-25 upright freezer 12 cubic feet	Biocold Scientific	\$3,000
	-25 upright freezer 23 cubic feet	Biocold Scientific	\$4,500
	-25 upright freezer 35cubic feet	Biocold Scientific	\$5,500
	-86 upright freezer 20 cubic feet	Cole-Parmer	\$21,000
	-86 upright freezer 25 cubic feet	Cole-Parmer	\$22,000
	-86 upright freezer 32 cubic feet	Cole-Parmer	\$26,000
Cryotanks	walk-in (8 x 8 x 7 ft.)	various restaurant suppliers	\$8,000
	storage racks and boxes, per cubic foot	Leigh Labs	\$370
Preservation Apparatus	Cryotankswith autofill and automonitor	Custom Biogenics Systems	\$23,000
	40000 vial model	Custom Biogenics Systems	\$24,000
Microscopy	46000 vial model	Custom Biogenics Systems	\$17,000
	Controlled Rate Freezer	Custom Biogenics Systems	\$7,000
DNA Analysis	Light / Digital Camera	Cole-Parmer	\$4,000
	Phase-Contrast	Cole-Parmer	\$80,000
	SEM	lab equip online	\$70,000
	TEM	lab equip online	\$40,000
	Immunofluorescent + camera	Zeiss	\$10,000
DNA Analysis	Pulse-Field Gel Electrophoresis	Amersham	\$4,000
	PCR Thermocycler	ABI	\$600,000
	DNA Sequencing / Restriction	Amersham	\$132,000
	Fragment Length Polymorphism	ABI	
Real-Time PCR analysis/detection			

Equipment	Manufacturer / Kind	Price Source	Price
	Nucleic Acid Prep-Station	ABI	\$22,000
	Standard DNA gel electrophoresis	Amersham	\$1,000
	DNA primer synthesis	ABI	\$180,000
Incubators	Incubators	Cole-Parmer	\$2,000
	walk-in temperature rooms	various restaurant suppliers	\$5,000
	CO2 incubators	Cole-Parmer	\$7,000
	Chilling incubator	Cole-Parmer	\$4,000
	Anaerobic incubator	lab equip online	\$5,000
Sterile Environments	8 ft. Laminar Flow Hood	Terra Universal	\$15,000
	BL2 Safety Cabinet	Terra Universal	\$15,000
	Anaerobic glove box	Cole-Parmer	\$18,000
Autoclaves	modern large upright autoclave	Alfa Sterilizers	\$80,000
	small benchtop steam autoclave	Alfa Sterilizers	\$20,000
Centrifuges	Microfuges, refrigerated	Cole-Parmer	\$6,000
	Large Centrifuges, refrigerated	Cole-Parmer	\$15,000
	medium size centrifuges	Cole-Parmer	\$2,000









Total		\$51,000	\$51,000	\$51,000	\$51,000	\$51,000	\$51,000
-------	--	----------	----------	----------	----------	----------	----------

**Appendix XIII Key Culture Collections Used During Survey****ATCC**

American Type Culture Collection

P.O. Box 1549

Manassas, VA 20108 USA

Webpage : <http://www.atcc.org/>

Holdings : bacteria, bacteriophage, cell lines, fungi, plants, protozoa, viruses

**BCCM**

The Belgian Coordinated Collections of Micro-organisms

OSTC - Wetenschapsstraat 8 Rue de la Science

B-1000 Brussels (Belgium)

Webpage : <http://www.belspo.be/bccm/index.htm>

Holdings : bacteria, fungi, plasmids

**CBS**

Centraalbureau voor Schimmelcultures

P.O.Box 85167

3508 AD Utrecht The Netherlands

Webpage : <http://www.cbs.knaw.nl/>

Holdings : bacteria, yeasts

**NCPV / UKNCC**

National Collection of Pathogenic Viruses

Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT

Webpage : <http://www.ukncc.co.uk/> and <http://www.camr.org.uk/>

Holdings : algae, bacteria, cell lines, fungi, protozoa

**NCTC / UKNCC**

National Collection of Type Cultures

Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT

Webpage : <http://www.ukncc.co.uk/>

Holdings : algae, bacteria, cell lines, fungi, protozoa

**UAMH**

University of Alberta Microfungus Collection & Herbarium

Devonian Botanic Garden

Edmonton, AB, Canada T6G 2E1

Webpage : <http://www.devonian.ualberta.ca/uamh/>

Holdings : fungi

### Appendix XIV Possible Sample Sources

Collection Name	WDCM #	V/B/F	Number of Strains Held	Cost / Strain		Possible Source	Online Catalog
				Government	Collection		
Belgian Coordinated Collections of Microorganisms	296	B	18000	?	?	Yes	Yes
Belgium Bacterial Culture Collection	400	B	3500	?	?	Yes	Yes
Belgium BCCM/IHEM	642	F	13000	\$57	\$57	Yes	No
Czech Animal Pathogens Institute	181	V	328	?	?	Possible?	No
Czech Collection of Fungi	182	F	1800	\$36	Trade	Yes	No
Czech Collection of Microorganisms	64	B	2400	\$43	Trade	Possible?	No
Czech Collection of Microorganisms	64	F	850	\$43	Trade	Yes	No
Czech National Collection of Type Culture	130	B	5500	?	Trade	Yes	No
Czech National Collection of Type Culture	130	V	650	?	Trade	Yes	No
Denmark IBTCulture Collection of Fungi	758	F	22000	?	Trade	Yes	No
Estonia Tartu Fungal Collection	821	F	2400	?	Trade	Yes	No
France Collection Francaise des Bacteries Phytopathogenes	629	B	3900	\$43	Trade	Yes	No
France Collection Nationale de Cultures de Microorganismes	174	V	194	?	?	Possible?	No
Germany Institute of Virology	429	V	120	?	?	Possible?	No
Germany Medical Culture Collection Marburg	418	B	3000	?	Trade	Yes	Yes
Hungarian National Collection of Medical Bacteria	258	B	3350	?	?	Yes	Yes
Hungary Collection of Animal Viruses	427	V	36	Free	Free	Yes	No
Indian Type Culture Collection	430	F	2500	?	?	Possible?	No
Japan Collection of Microorganisms	567	B	6400	?	?	Yes	Yes
Japan Collection of Microorganisms	567	F	1200	?	?	Possible?	No
Korean Collection for Type Cultures	597	B	5000	?	Trade	Possible?	No
Korean Collection for Type Cultures	597	F	400	?	Trade	Yes	No
NCCB, the Netherlands Culture Collection of Bacteria	797	B	10000	?	?	Yes	Yes
Netherlands Centraalbureau voor Schimmelcultures	133	F	40500	\$64	Trade	Yes	No
New Zealand Reference Culture Collection, Medical Section	457	B	4000	?	?	Yes	Yes
Norway Medical Microbiological Laboratory	432	V	1900	?	?	Possible?	No
Polish Collection of Microorganisms	106	B	2200	?	?	Possible?	No
Sweden Culture Collection, University of Goteborg	32	B	40000	\$86	\$86	Yes	Yes
UK National Collection of Pathogenic Fungi	184	F	1300	?	?	Possible?	No
UK National Collection of Pathogenic Viruses	814	V	180	?	?	Yes	Yes
UK National Collection of Type Cultures	154	B	5000	\$107	\$107	Yes	Yes

Note - information on contacting culture collections, accessing catalogs etc can be found at the WDCM webpage:  
<http://www.wdcm.org/hpcc.html>

Information for collections above may also be searched using the WDCM identity number listed above



**DOCUMENT CONTROL DATA**

(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)

1. ORIGINATOR (the name and address of the organization preparing the document. Organizations for who the document was prepared, e.g. Establishment sponsoring a contractor's report, or tasking agency, are entered in Section 8.)  Dr. Donald J. Netolitzky, Edmonton, Alberta			2. SECURITY CLASSIFICATION (overall security classification of the document, including special warning terms if applicable)  UNCLASSIFIED		
3. TITLE (the complete document title as indicated on the title page. Its classification should be indicated by the appropriate abbreviation (S, C or U) in parentheses after the title).  Feasibility Study of Canadian Pathogen Collections					
4. AUTHORS (Last name, first name, middle initial. If military, show rank, e.g. Doe, Maj. John E.)  Netolitzky, Dr. Donald J.					
5. DATE OF PUBLICATION (month and year of publication of document)  September 2003		6a. NO. OF PAGES (total containing information, include Annexes, Appendices, etc) 104		6b. NO. OF REFS (total cited in document)  41	
7. DESCRIPTIVE NOTES (the category of the document, e.g. technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)  Final Contract Report					
8. SPONSORING ACTIVITY (the name of the department project office or laboratory sponsoring the research and development. Include the address.)  DRDC Suffield					
9a. PROJECT OR GRANT NO. (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.)			9b. CONTRACT NO. (If appropriate, the applicable number under which the document was written.)  W7702-03P159/001/EDM		
10a. ORIGINATOR'S DOCUMENT NUMBER (the official document number by which the document is identified by the originating activity. This number must be unique to this document.)			10b. OTHER DOCUMENT NOS. (Any other numbers which may be assigned this document either by the originator or by the sponsor.)  CR-2003-133		
11. DOCUMENT AVAILABILITY (any limitations on further dissemination of the document, other than those imposed by security classification)  <input checked="" type="checkbox"/> Unlimited distribution <input type="checkbox"/> Distribution limited to defence departments and defence contractors; further distribution only as approved <input type="checkbox"/> Distribution limited to defence departments and Canadian defence contractors; further distribution only as approved <input type="checkbox"/> Distribution limited to government departments and agencies; further distribution only as approved <input type="checkbox"/> Distribution limited to defence departments; further distribution only as approved <input type="checkbox"/> Other (please specify):					
12. DOCUMENT ANNOUNCEMENT (any limitation to the bibliographic announcement of this document. This will normally corresponded to the Document Availability (11). However, where further distribution (beyond the audience specified in 11) is possible, a wider announcement audience may be selected).					

13. ABSTRACT (a brief and factual summary of the document. It may also appear elsewhere in the body of the document itself. It is highly desirable that the abstract of classified documents be unclassified. Each paragraph of the abstract shall begin with an indication of the security classification of the information in the paragraph (unless the document itself is unclassified) represented as (S), (C) or (U). It is not necessary to include here abstracts in both official languages unless the text is bilingual).

An important scientific capability of many nations is a national culture collection of a wide variety of microorganisms used both in research as well as important reference standards in microbial identification. In the past, Canadian researchers have relied heavily on the close proximity and ease of access to the American Type Culture Collection as well as close links to counterparts in US laboratories. With the implementation of the Homeland Security Act we now find ourselves in a position where it is difficult if not impossible to obtain cultures related to bioterrorism research from the US. Fortunately, the US is not the only place in which we might obtain microorganisms from a culture collection. At present we still have the option to obtain materials from the UK or one of many other national culture collections around the world. Even small nations such as Belgium have their own national culture collection. Canada does not. While it is still possible to obtain these materials elsewhere, there is no certainty that this situation will last indefinitely. It may well turn out that other nations may also go the route of increasing security for biological agents which might, eventually, leave us in a poor position with little or no access to a variety of unique microorganisms needed for research related to defence or national security issues.

This report is a feasibility study of establishing a national culture collection that looks at the current situation of culture collections in Canada and provide recommendations in several areas that provide options filling some deficiencies and creation of a national collection. Canada does not have a true pathogen collection encompassing all the significant medical and zoonotic pathogens. The creation of a "Canadian Pathogen Collection" is an option that would deal with this deficiency. The report suggests that such a collection would be held jointly by DRDC Suffield and the National Microbiology Lab in Winnipeg. An important part of this collection would be the creation of a shared and extensive inventory software application accessible to both over the internet. This application is a crucial resource required to allow management of collection resources and provide a mechanism to find strains in the collection and make requests. Information on individual strains should be as detailed and extensive as possible. As noted in the report, "A poorly described strain, or one that is difficult to find, effectively does not exist."

Finally, a recommendation towards the creation of a national collection described as the "Canadian Microorganism Collection Network" is provided. Rather than having a single centralized collection this recommendation is one that would link existing culture collections by electronic means, using the software application mentioned above. A collection network is cost effective and promises to correct long-standing deficiencies in Canada's culture collection strategy. The network itself would hold no microorganisms, but would serve as an umbrella organization for new and existing culture collections in Canada.

14. KEYWORDS, DESCRIPTORS or IDENTIFIERS (technically meaningful terms or short phrases that characterize a document and could be helpful in cataloguing the document. They should be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location may also be included. If possible keywords should be selected from a published thesaurus, e.g. Thesaurus of Engineering and Scientific Terms (TEST) and that thesaurus-identified. If it is not possible to select indexing terms which are Unclassified, the classification of each should be indicated as with the title.)

culture collection  
feasibility study  
biological defence