Advances in technologies for detection of infectious diseases

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Pathogens detection

Humans
Animals
Plants
Environment
Food and feed
Pathogen detection applications

Pathogen detection has become an important part of research in many fields like:

- Biodefense
- Animal health care
- Food safety
- Diagnostics
- Pathology
- Clinical research
- Forensics
- Drug discovery
For biodefense, accurate analytical techniques for discovering pathogenic agents are needed.

Health care community uses pathogen detection to develop various diagnostic tests that are rapid, reliable and highly sensitive for effective control and treatment of diseases.

In diagnostics, the technique is employed to detect or identify infectious agents, toxins, parasites, metabolic disorders, and genetic susceptibility or resistance.
Challenges in pathogen detection

The predominant techniques currently used to identify microbial pathogens:

- Conventional clinical microbiology monitoring approaches that are well established suffer from a number of considerable drawbacks.
- Standard culture and susceptibility tests permit pathogen identification but is laborious, time-consuming, expensive and require labile natural products.
- The tests that are routinely utilized for pathogen identification do not directly characterize virulence factors.
- Problems with managing large numbers of environmental or clinical samples.
# Pathogens in the most common bacterial infections

<table>
<thead>
<tr>
<th>Pathogens in the most common bacterial infections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sepsis</strong></td>
</tr>
<tr>
<td>Gram-negative bacteria: <em>E. coli</em>, <em>Klebsiella sp.</em>, <em>Pseudomonas aeruginosa</em>, other <em>Enterobacteriaceae</em>, <em>Salmonella sp.</em>, <em>Bacteroides sp.</em></td>
</tr>
<tr>
<td>Gram-positive bacteria: <em>S. aureus</em>, coagulase-negative staphylococci, <em>Enterococci</em>, non-hemolytic streptococci, pneumococci</td>
</tr>
<tr>
<td><strong>Bacterial endocarditis</strong></td>
</tr>
<tr>
<td>Acute endocarditis: <em>S. aureus</em>, <em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td>Subacute endocarditis: non-hemolytic streptococci, <em>Enterococci</em>, coagulase-negative staphylococci (especially in infections on artificial heart valves)</td>
</tr>
<tr>
<td><strong>Bacterial infections of the central nervous system</strong></td>
</tr>
<tr>
<td><strong>Meningitis</strong></td>
</tr>
<tr>
<td>Acute purulent meningitis: pneumococci, <em>N. meningitidis</em>, <em>Haemophilus influenzae</em>, <em>E. coli</em>, group B <em>Streptococcus</em> (GBS), <em>S. aureus</em>, <em>S. epidermidis</em>, group A <em>Streptococcus</em> (GAS)</td>
</tr>
<tr>
<td>Chronic lymphocytic meningitis: <em>M. tuberculosis</em>, <em>Listeria</em></td>
</tr>
<tr>
<td>Differentiation: <em>Leptospira</em>, <em>Cryptococcus neoformans</em> (HIV patients!), <em>T. gondii</em>, amoeba (<em>Naegleria sp.</em>)</td>
</tr>
<tr>
<td><strong>Subdural empyema</strong></td>
</tr>
<tr>
<td><em>Streptococcus</em>, <em>Staphylococcus</em>, <em>Pneumococci</em>, <em>Haemophilus influenzae</em>, <em>Enterobacteriaceae</em>, <em>Pseudomonas sp.</em></td>
</tr>
<tr>
<td><strong>Brain abscess</strong></td>
</tr>
<tr>
<td><em>S. aureus</em>, <em>Enterobacteriaceae</em>, <em>Pneumococci</em>, <em>Haemophilus influenzae</em>, <em>Bacteroides sp.</em>, <em>Cryptococcus neoformans</em> in immunosuppressed patients</td>
</tr>
<tr>
<td><strong>Conjunctivitis</strong></td>
</tr>
<tr>
<td><em>Pneumococci</em>, <em>S. aureus</em>, <em>Haemophilus influenzae</em>, less frequently <em>Enterobacteriaceae</em>, gonococci</td>
</tr>
<tr>
<td><strong>Otitis media</strong></td>
</tr>
<tr>
<td><em>Pneumococci</em>, <em>Haemophilus influenzae</em>, <em>Moraxella catarrhalis</em>, <em>Pseudomonas sp.</em></td>
</tr>
</tbody>
</table>
# Pathogens in the most common bacterial infections

<table>
<thead>
<tr>
<th>Bacterial respiratory tract infection</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinusitis or rhinosinusitis</td>
<td><em>Pneumococci, Haemophilus influenzae, S. aureus, group A Streptococcus</em> (GAS), <em>Moraxella catarrhalis, Pseudomonas sp.</em>, Enterobacteriaceae, anaerobes (odontogenic infection)</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>Group A <em>Streptococcus</em> (GAS), less frequently <em>Corynebacterium diphtheriae</em>, gonococci</td>
</tr>
<tr>
<td>Acute laryngitis and tracheitis (croup)</td>
<td><em>Haemophilus influenzae</em>, less frequently <em>Corynebacterium diphtheriae</em>, <em>Mycoplasma pneumoniae</em></td>
</tr>
<tr>
<td>Acute bronchitis</td>
<td><em>Mycoplasma pneumoniae</em>, <em>Bordetella pertussis</em>, <em>Chlamydia psittaci</em>, <em>Chlamydia pneumoniae</em></td>
</tr>
</tbody>
</table>
| Pneumonia                                  | Lobar pneumonia or bronchopneumonia: *pneumococci, S. aureus, Haemophilus influenzae, Enterobacteriaceae, Pseudomonas sp.*
|                                            | Interstitial pneumonia: *Mycoplasma pneumoniae, Legionella, Chlamydia pneumoniae* |
|                                            | Differentation: *Pneumocystis carinii* in immunosuppressed patients, in the aspiration pneumonia also anaerobes |
### Pathogens in the most common bacterial infections

<table>
<thead>
<tr>
<th>Bacterial respiratory tract infection</th>
<th>E. coli, other Enterobacteriaceae, Pseudomonas sp., Enterococci, S. saprophyticus, Chlamydia trachomatis, Mycoplasma, less frequently Gonococci, Mycobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastroenteritis</td>
<td>Bacteria of the Schigella genus, Bacteria of the Salmonella genus, enteric pathogens E. coli, Yersinia, Campylobacter jejuni, Clostridium difficile, Vibrio cholerae, action of bacterial toxins produced by S. aureus, Clostridium botulinum and Bacillus cereus</td>
</tr>
<tr>
<td>Skin and wound infections</td>
<td>S. aureus, group A Streptococcus (GAS), Pseudomonas aeruginosa, Enterobacteriaceae, after animal bites also Pasteurella multocida</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>S. aureus, less frequently Haemophilus influenzae, group A Streptococcus (GAS), Pseudomonas aeruginosa, Enterobacteriaceae and bacteria of the Salmonella and Mycobacteria genus</td>
</tr>
</tbody>
</table>
Airborne Pathogen Database - Fungi

Aspergillus spp.
Mucor plumbeus
Blastomyces dermatitidis
Micropolyspora faeni
Cladosporium spp.

Absidia corymbifera
Cryptococcus neoformans
Coccidioides immitis
Thermoactinomyces vulgaris
Helminthosporium

Rhizopus stolonifer
Histoplasma capsulatum
Penicillium spp.
Alternaria alternata
Stachybotrys spp.
Detecting the presence of bacteria

- Direct: the observation of the presence of infectious agents, components or products, such as exotoxin.
- Indirect: detection of antibodies produced in the course of infectious diseases against microorganisms and their antigenic determinants.
Direct detection of the presence of bacteria

Classical methods:
- Microscopic examination of fresh material, direct preparation (stained)
- In vitro culture and identification of microbial species. The culture is still considered as the „gold standard”.
- Antibiogram to determine antibiotic resistance.

New methods:
- Demonstration of the presence of antigen by immunological methods (agglutination, precipitation, luminiscence, immunofluorescence)
- Molecular probes
- Amplification of nucleic acids
Quick detection of periodontitis pathogens

30-50% of population suffer from periodontitis, an inflammation that can lead to the loss of teeth if left untreated. A new diagnostic platform enables the pathogens to be detected quickly, enabling dentists to act swiftly to initiate the right treatment.

Of the estimated 700 species of bacteria found in the mouth cavity, there are only eleven that are known to cause periodontal disease in particular; of these, some are deemed to be severely pathogenic.

*Peptostreptococcus sp.*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Actinomyces sp*, *Fusobacterium necrophorum*, *Prevotella denticola*, *Capnocytophaga sp.*, *Eikenella corrodens*, *Prevotella oralis*
Quick detection of periodontitis pathogens

Conventional bacterial analysis using microbial culture carries the risk of bacteria being killed as soon as they come into contact with oxygen. A lab-on-a-chip module called Parodontitis-Chip will allow dentists and medical labs to prepare samples quickly and then analyze the bacteria. All the steps in the process – the duplication of DNA sequences and their detection – take place directly on the platform, which consists of a disk-shaped microfluidic card that is around six centimeters in diameter.
The presence of bacteria *Porphyromonas gingivalis* in atherosclerotic plaques and vascular wall specimens.

People with periodontal disease are almost twice as likely to have coronary artery disease. The presence of common problems in the mouth, including gum disease (gingivitis), cavities, and missing teeth, were as good at predicting heart disease as cholesterol levels.
Genotyping of pathogens commonly encountered in the clinic

Patients who have an infection (i.e., multiplication of an infectious agent in their tissues, resulting in subclinical or clinical illness) or colonization (i.e., presence of microorganisms without tissue invasion or injury) serve as reservoirs for these microorganisms. The risk factors for colonization include such factors as age, severity of illness and use of antibiotics.

Electrophoretic separation of PCR products with primers 16S5EF, 16SV89 and 16SISR derived from the DNA of bacteria, 42 (Escherichia coli) (lanes 1,3,5) and 43 (Proteus mirabilis) (lanes 2, 4, 6), respectively.
Genotyping of pathogens commonly encountered in the clinic

Sequencing of the PCR product obtained using the forward primer 16SISR performed for sample 43 (*Proteus mirabilis*).
Indirect detection of the presence of bacteria

Serological identification of antibodies by:

- Immunoprecipitation
- Agglutination
- Complement fixation
- RIA
- ELISA
- Capture assay
- Immunofluorescence
- Hemagglutination inhibition assay
- Neutralization test

Determination is required in cases when it is difficult to demonstrate directly the infectious agent.
Anthrax detection

The sensor measures the presence of dipicolinic acid (DPA). The sensor consists of a glass plate to which DPA-sensitive receptors have been attached. When the receptors are brought into contact with anthrax spores, the DPA binds with them.

DPA-bonded receptors will absorb this light and emit blue light, whereas receptors that have no DPA bonding will emit red light. By measuring the ratio of red to blue light in a sample, it is possible to determine the concentration of anthrax spores.
Anthrax detection

Recent advances in detection and identification techniques could prove to be an essential component in the defense against biological attacks.

Sequence based such as pyrosequencing, which has the capability to determine short DNA stretches in real-time using biotinylated PCR amplicons, has potential biodefense applications.

Using markers from the virulence plasmids (pXO1 and pXO2) and chromosomal regions, it was possible to demonstrate the power of this technology in the rapid, specific and sensitive detection of *B. anthracis* spores in food matrices including milk, juice, bottled water, and processed meat.
Alignments of the sequence data collected during pyrosequencing assays for the downselected targets (gerXB for pXO1, acpB for pXO2, and prophage lambda3 for the chromosome).
Transfer of operon responsible for conversion of glycerol into 1,3-propanediol between pathogenic bacteria *Klebsiella pneumoniae* to non-pathogenic bacterial strain of *E.coli*
Transfer of metabolic pathways from pathogens

E. coli BL21 strain modified with metabolic pathway from pathogenic *Citrobacter freundii* (colony no. 1527)
Virus detection

Virus detection is very important in many fields such as

- Health,
- Food production,
- Biotechnology processes,
- Plant protection and
- Detection of potential biological weapons attack

In food production, it is necessary to precisely detect viruses in very complex samples, often with very small water contents. The detection of biological warfare attack is necessary to detect small amounts of virus circulating in the air.
## Clinically important virus families and species

<table>
<thead>
<tr>
<th>Family</th>
<th>Baltimore group</th>
<th>Important species</th>
<th>Envelopment</th>
<th>Virion shape</th>
<th>Replication site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviridae</td>
<td>Group I</td>
<td>Adenovirus</td>
<td>Non-enveloped</td>
<td>Icosahedral</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Herpesviridae</td>
<td>Group I</td>
<td>Herpes simplex, type 1, Herpes simplex, type 2, Varicella-zoster virus, Epstein-barr virus, Human cytomegalovirus, Human herpesvirus, type 8</td>
<td>Enveloped</td>
<td>Complex</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Papillomaviridae</td>
<td>Group I</td>
<td>Human papillomavirus</td>
<td>Non-enveloped</td>
<td>Icosahedral</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Polyomaviridae</td>
<td>Group I</td>
<td>BK virus, JC virus</td>
<td>Non-enveloped</td>
<td>Icosahedral</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Poxviridae</td>
<td>Group I</td>
<td>Smallpox</td>
<td>Enveloped</td>
<td>Complex</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Hepadnaviridae</td>
<td>Group VII</td>
<td>Hepatitis B virus</td>
<td>Enveloped</td>
<td>Icosahedral</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Paroviridae</td>
<td>Group II</td>
<td>Human bocavirus, Parovirus B19</td>
<td>Enveloped</td>
<td>Icosahedral</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Astroviridae</td>
<td>Group IV</td>
<td>Human astrovirus</td>
<td>Non-enveloped</td>
<td>Icosahedral</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Caliciviridae</td>
<td>Group IV</td>
<td>Norwalk virus</td>
<td>Non-enveloped</td>
<td>Icosahedral</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Picornaviridae</td>
<td>Group IV</td>
<td>coxsackie virus, hepatitis A virus, poliovirus, rhinovirus</td>
<td>Non-enveloped</td>
<td>Icosahedral</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Coronaviridae</td>
<td>Group IV</td>
<td>Severe acute respiratory syndrome virus</td>
<td>Enveloped</td>
<td>Helical</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Flaviviridae</td>
<td>Group IV</td>
<td>Hepatitis C virus, yellow fever virus, dengue virus, West Nile virus</td>
<td>Enveloped</td>
<td>Icosahedral</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Togaviridae</td>
<td>Group IV</td>
<td>Rubella virus</td>
<td>Enveloped</td>
<td>Icosahedral</td>
<td>Cytoplasm</td>
</tr>
</tbody>
</table>
## Clinically important virus families and species

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</tr>
</thead>
<tbody>
<tr>
<td>Hepeviridae</td>
<td>Group IV</td>
<td>Hepatitis E virus</td>
<td>Enveloped</td>
<td>Icosahedral</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Retroviridae</td>
<td>Group VI</td>
<td>Human immunodeficiency virus (HIV)</td>
<td>Enveloped</td>
<td>Icosahedral</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Orthomyxoviridae</td>
<td>Group V</td>
<td>Flu virus</td>
<td>Enveloped</td>
<td>Helical</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Arenaviridae</td>
<td>Group V</td>
<td>Guanarito virus, Junin virus, Lassa virus, Machupó virus, Sabiá virus</td>
<td>Enveloped</td>
<td>Helical</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Bunyaviridae</td>
<td>Group V</td>
<td>Crimean-Congo hemorrhagic fever virus</td>
<td>Enveloped</td>
<td>Helical</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Filoviridae</td>
<td>Group V</td>
<td>Ebola virus, Marburg virus</td>
<td>Enveloped</td>
<td>Helical</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Paramyxoviridae</td>
<td>Group V</td>
<td>Measles virus, Mumps virus, Parainfluenza virus, Respiratory syncytial virus, Human metapneumovirus, Hendra virus, Nipah virus</td>
<td>Enveloped</td>
<td>Helical</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Rhabdoviridae</td>
<td>Group V</td>
<td>Rabies virus</td>
<td>Enveloped</td>
<td>Helical, bullet shaped</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Unassigned</td>
<td>Group V</td>
<td>Hepatitis D</td>
<td>Enveloped</td>
<td>Spherical</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Reoviridae</td>
<td>Group III</td>
<td>Rotavirus, Orbivirus, Coltivirus, Banna virus</td>
<td>Non-enveloped</td>
<td>Icosahedral</td>
<td>Cytoplasm</td>
</tr>
</tbody>
</table>
Airborne Pathogen Database - Viruses

- Orthomyxoviridae - Influenza
  - Arenavirus - Junin
  - Filovirus - Ebola
  - Arenavirus - Lassa
  - Filovirus - Marburg
  - Picornoviridae - Echovirus
  - Hantaviruses
  - Picornoviridae - Rhinoviruses
  - Morbillivirus
  - Coronavirus
  - Paramyxovirus
  - Coxsackievirus
  - Respiratory Syncytial Virus
  - Togavirus
  - Adenoviruses
  - Parvovirus B19
  - Parainfluenza
  - Poxvirus - Variola
  - Reoviruses
  - Arenavirus - Machupo
  - Varicella-zoster
Direct detection of the presence of viruses

- Direct IFA, e.g. content of bubbles in the case of *Herpes zoster*
- Serological detection of virus antigen (ELISA-RIA, e.g., Hb5Ag)
- Molecular diagnostics based on DNA probes (more often used)
- PCR (more often used)
- Virus propagation (replaced by molecular methods), in the cell culture and incubated chicken embryos; in samples of animals, such as newborn mouse (a very expensive method used when other failures)
- Electron microscopy (not very useful in routine diagnostics is used only in specialized laboratories)
Indirect detection of the presence of viruses

Demonstration in the patient's serum specific antibodies against viruses. The most important methods for the determination of antibody titers are the ELISA and IFA. Immunoblotting and determination of antibody avidity are used increasingly.
Methods based on DNA amplification

- PCR, polymerase chain reaction, amplification of DNA and RNA sequences
- NASBA, nucleic acid sequence based amplification used to amplify RNA sequences
Polymerase chain reaction

This method has been used successfully in the detection of an increasing number of viruses, such as swine vesicular disease virus, human metapneumovirus, West Nile virus, hepatitis B and C virus, herpes simplex virus, human and bovine respiratory syncytial virus, Norwalk virus, influenza viruses, Nipah virus, orthopoxviruses, Rift Valley fever virus, cytomegalovirus and many others.
Direct detection of the presence of viruses

A) DNA amplimers of PERV-A, PERV-B and PERV-C integrated into the host genome (PCR envPERV). M, DNA size marker (pBR322/HaeIII).

B) PERV DNA fragments integrated into the host genome (long-PCR). M, DNA size marker (lambda/BstPI). Lanes 1-4, Detection of PERV DNA integrated into the genome of pigs in NRIAP breeding flocks.
Identification of Papillomavirus type 3 at the dairy cows

Infection spread easily by chafing of healthy and infected animals against the same objects. Other objects used in routine animal care like pliers for the tattoo, the needles and surgical tools as well as mosquitoes and ticks contribute to infection spread among the cattle’s.

Electrophoresis of PCR products obtained after amplification of the DNA fragments encoding the BPV-3 capsid protein. M, DNA size marker (pBR 322/HaeIII); lanes 2-17, products of amplification of DNA samples. BPV-3 viral sequences present in lanes 1-4.
Methods based on direct observation of viral particles

- Electron microscopy, EM
- Electron tomography, ET
- Scanning electron microscopy, SEM
- Atomic force microscopy, AFM
- Fluorescence microscopy, FM
Methods based on direct observation of viral particles

Electron microscopy, EM

One of the main advantages of using EM for viral diagnosis is that it does not require organism-specific reagents for recognizing the pathogenic agent. Because it can be a rapid procedure, EM is on the front line in surveillance of viruses that might be used by terrorists. [http://www.bt.cdc.gov/agent/smallpox/lab-testing/pdf/em-rash-protocol.pdf](http://www.bt.cdc.gov/agent/smallpox/lab-testing/pdf/em-rash-protocol.pdf)
Methods based on direct observation of viral particles

Electron microscopy, EM

Negative stain of a small naked (poliovirus) and large naked (adenovirus) icosahedral virus

Methods based on direct observation of viral particles

Electron microscopy, EM

Negative stain of an enveloped virus with clear surface projections (influenza B virus) and virus with icosahedral nucleocapsid (herpesvirus).

Methods based on direct observation of viral particles

Electron microscopy, EM

Thin section of a paracrystalline array of a naked DNA virus (adenovirus) in the nucleus of an infected cell and a naked RNA virus (Nodamura virus) produced in the cytoplasm.

Methods based on direct observation of viral particles

Electron tomography, ET

(A) Simian immunodeficiency virus viewed frozen hydrated and unstained in a cryo 300-kV transmission electron microscope. (B) Four 1-nm-thick slices from a tomogram. (C) Computer-generated 3D reconstruction of viral particle. Bars, 50 nm. Magnification, ×100,000.

Methods based on direct observation of viral particles

Scanning electron microscopy, SEM

Scanning EM image of HIV budding from the cell surface of a lymphocyte (arrow). Bar, 100 nm. Magnification, ×50,000.

Methods based on direct observation of viral particles

Atomic force microscopy, AFM

Herpes simplex virus adsorbed onto silanized glass slide measured in buffer solution; virus substructure can be easily resolved. 3D topography. Scan size 300x300 nm, Z-range 150 nm, image in closed-loop.

Sample courtesy Dr Wouter Roos, Vrije Universiteit Amsterdam, the Netherlands
Multiple sclerosis associated retrovirus (MSRV) has been linked to MS pathogenesis, it belongs to the human endogenous retrovirus-W family and produces extracellular virions, found in plasma and CSF of MS patients.

MSRV sequences **pol**, **gag** and **env** in MS patients detected by FISH

MSRV sequences in interphase nuclei of MS patients:  
A) **pol**, B) **gag**, C) **env**.

Courtesy of M. Zawada
Detection of infectious agents using PCR

- Humans
- Dogs
- Cats
- Pigs
- Horses
- Birds
There are many requirements

- Sensitive but resistant to false positive results
- Fast
- Inexpensive
- Capable of full automation
Airborne pathogen control technologies

Current
- Isolation systems
- Air filtration
- Ultraviolet irradiation
- Outdoor air purging
- Electrostatic precipitation
- Negative air ionization
- Vegetation

Developmental
- Photocatalytic oxidation
- Air ozonation
- Carbon adsorption
- Passive solar exposure
- Ultrasonic atomization
- Microwave atomization
- Pulsed light
Obtaining the virus imitating particles and evaluation of barrier properties of filters

PZ III. Bionanofibres as virus barriers.
PZ III.3. Obtaining of nonapathogenic gene constructs for evaluation of filters.
Nagoya Protocol on Access to Genetic Resources

- Exchange of cells and microorganisms between laboratories in various countries
- Purchase of cells and microorganism from specialised companies
- Order to prepare gene constructs in biotech centres
Agents considered for weaponization

Bacteria:
- *Bacillus anthracis*
- *Brucella spp.*
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- *Chlamydophila psittaci*
- *Coxiella burnetii*
- *Francisella tularensis*
- *Rickettsia prowazekii* and *Rickettsia rickettsii*
- *Shigella spp.*
- *Vibrio cholerae*
- *Yersinia pestis*
Agents considered for weaponization

Viruses:
- *Bunyaviridae* (especially Rift Valley fever virus)
- Ebola virus
- *Flaviviridae* (especially Japanese encephalitis virus)
- Machupo virus
- Marburg virus
- Variola virus
- Yellow fever virus
Lack of concern about the dual use potential of new developments in life sciences;
Lack of a proper sense of responsibility in safeguarding against the potential misuse;
Lack of effective and systematic regulatory measures;
Lack of dissemination of knowledge on dual use potential of scientific breakthroughs in the life sciences.
The Polish Academy of Sciences and IAP together with the members of the BWG are planning the conference on advances in surveillance, detection and diagnosis of infectious diseases. The conference will be held in Warsaw on December 6. Details will be released soon.