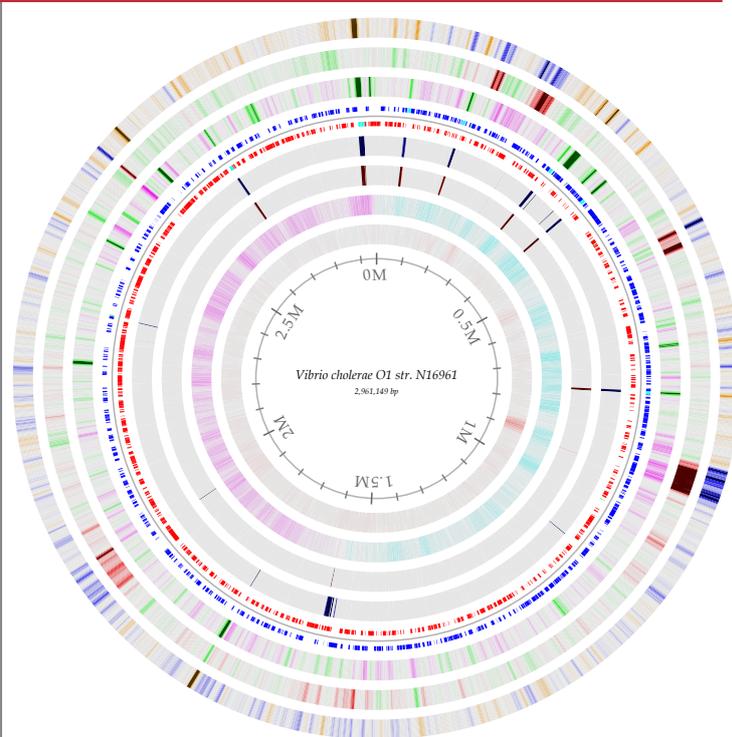


Perspectives of a global, real-time microbiological genomic identification system - implications for national and global detection and control of infectious diseases

Consensus report of an expert meeting 1-2 September 2011, Bruxelles, Belgium



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National Food Institute
Technical University of Denmark

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1. INTRODUCTION

The National Food Institute, Danish Technical University, organized an expert meeting on microbiological genomic identification systems 1-2 September 2011 in Bruxelles, Belgium.

The purpose of the meeting was to assemble a group of imminent scientists and managers to debate the necessity, feasibility, benefit and broader governance requirements of a global system for identification of microorganisms based on genomic information. The background for the organization of the meeting was the following projections:

- Globally up to ½ billion microbiological isolates are characterized every year in presently very diverse and expensive typing systems involving serology, phenotypic testing and different types of DNA fingerprinting techniques, all of which could be replaced by total genome sequencing (for both bacteria and virus).
- An increasing number of human infectious diseases have a global epidemiology (e.g. SARS, avian flu, influenza, *Salmonella* to name a few). Therefore rapid detection and identification of microbial agents, enabling timely response and control are crucial capacities to avoid or control global spread.
- It is likely that in 5 to 10 years all clinical microbiological laboratories will have a DNA sequencer in use on a daily basis and within that timeframe the costs for a complete bacterial genome sequence might be less than 100 EURO (or US\$). The capacity to exchange – and manage - large data quantities over web-based systems has likewise increased dramatically over recent years, enabling the potential creation of global databases consisting of DNA-codes of all relevant microbiological strains
- Most likely the future limiting factor will not be the cost of whole genome sequencing, but how to assemble, process and handle the large amount of data in a standardized way that will make the information useful, especially for diagnostics and surveillance.

Twenty-five participants, including twelve speakers, attended the meeting. The complete participants list is available as Annex 1. The agenda can be found in Annex 2. All presentations in pdf files can be found at: <http://www.food.dtu.dk/Default.aspx?ID=24273>

The participants were welcomed by Jørgen Schlundt, deputy director of the National Food Institute of the Danish Technical University. Dr. Schlundt was accepted by the meeting as chair of the deliberations, while Dr. Rene Hendriksen and Professor Frank Møller Aarestrup were accepted as rapporteurs.

2. TECHNICAL DELIBERATIONS

A. Present Potential and Perspectives

The discussions were informed by four presentations:

- A vision and present initiatives for the use of Whole Genome Sequencing (J. Schlundt)
- Presenting the Danish Center for Genomic Epidemiology (F.M. Aarestrup)
- Data technology possibilities – database possibilities (O. Lund)
- The global landscape relative to gene sequencing (M. Gilmour and P. Keim)

The ensuing discussions suggested that global detection and control using whole genome sequence typing (WGST) will require global standards in terms of nomenclature and precisely how to analyze the sequence data. It was suggested that sequencing will gain the same success as PFGE (Pulsed-Field Gel Electrophoresis) due to the rapid spread of a generic methodology.

There was a general acceptance that we are presently at the very beginning of the era of using WGST technology. Currently, there is a lack of high quality data suggesting a need for more data concerning all pathogens to determine future cut-offs. One solution could be the use of special nomenclature including a clear and globally agreed upon clone concept.

It was strongly emphasized that the initiation of a WGST database system does not depend on the final sequencing of all microorganisms, but data quality will be crucial to avoiding typing errors that at the moment are a huge problem for future analyses. The quality in GenBank would not seem to be adequate and could cause too many doubtful results. The process of retrieving new high quality data will be an ongoing process which needs to be adjusted over time. Several WGST studies have used different analyses tools, such as phylogenetic trees. Additionally, spatial and temporal analysis combined with WGST will be important for the future improved understanding of the epidemiology of all pathogens. However, the GenBank model remains important in providing leadership for WGS annotation and curation, and will be crucial moving forward towards an open and fully accessible WGS database populated with sequences from around the world.

In relation to data transport in support of the future WGST database and platform, the present Internet speed is too low, client computers are too slow, and the host capacity is currently limited. Since there might be some limitation in host capacity at this stage, a possibility could be to link several databases. However, two important issues need to be addressed; interface and microbiology nomenclature. It is estimated that the cost of building a new database hosting all future sequence data would be approx. 25mill USD. This estimation carries significant uncertainties and only refers to the set-up of the database per se. To facilitate a faster process regarding the assembly of the reads, this could be performed locally in comparison to a global database facilitating this part. This might create more fragmented sequence data. However, the pace of the technology development is presently so high that one possibility could be that future sequencers might have in-built assemblers thus resolving this issue.

Building a global system sharing often sensitive data will create barriers, one of which might be the willingness of sharing sequence data and the associated metadata. At this point, a clear description of the explicit purposes for sharing data needs to be agreed by relevant partners and stakeholders. Sensitive data have in the past not always been shared in a timely fashion. In the future, sequence data-bases need to have open access to serve as an early warning system to detect and control emerging infectious pathogens with incorporated “surveillance flag issues”. It should be realized that relevant WGST data might also be attractive for any industry searching for solutions to disease detection and control (including vaccine production etc.). However, important privacy issues concerning future data mining potential clearly exist.

B. Global policy context

The discussions were informed by four presentations:

- International Health Regulations – the need for sharing data (D. Lo-Fo-Wong)
- International perspectives – laboratory links (M. Koopmans)
- CDC’s Global Disease Detection Program (J. Sobel)
- Potential for innovative solutions in developing country settings (P. Keim)

The updated WHO International Health Regulations (IHR, 2005) now require all Member States of WHO to share information about public health events of international concern. IHR thus enables the sharing of relevant

data between countries. It would make sense to use the IHR regulations also to enable the sharing of relevant genomic data during potential public health events. However, a number of generic objections to full data sharing can be foreseen. A number of these have already been described in the EU project GESTURE (<http://ec.europa.eu/eahc/projects/database.html?prjn0=20084153>). The output report of the GESTURE project describes, amongst other things, a useful template for a code of conduct for data sharing. In doing so, it would be important to consider specifically those issues relevant to the situation in developing countries.

With the rapid pace of the development of the WGST technology, the Global Disease Detection programme (GDD) (<http://www.cdc.gov/globalhealth/gdder/gdd/>) hosted by the Centers for Disease Control and Prevention in the USA might soon (few years) use state-of-the-art sequencers, thus enabling the sharing of WGST data from many sources in several regions of the world. Today, GDD does not conduct any laboratory training in WGST as all training is facilitated by PulseNet (<http://www.cdc.gov/pulsenet/>) and the WHO Global Foodborne Infections Network (GFN) (<http://www.who.int/gfn/en/>). These networks are only sharing data according to rules laid down in agreements with the Ministries of Health in the affected countries. Thus, any sequence platform related to these networks would have to build on agreements with the Ministries of Health involved.

It is a pre-requisite for the successful implementation of new technologies for surveillance purposes that they are backwards compatible to ensure so that historical trends may be compared with current ones. In the future, it might be easier to perform sequencing compared to conventional culture resulting in an important leap-frog possibility for developing countries. The whole genome contains all the information needed for identification and comparison purposes. In moving forward it should be recognized that PFGE databases are highly valuable resources, meaning that backwards comparability to these would be beneficial for WGST databases.

C. What to do – and how to do it?

The discussions were informed by five presentations:

- Dealing with outbreaks – sequencing as an epidemiological tool (D. Harmsen)
- Future potential of Laboratory Networking (A. Kroneman)
- A world without culture isolates (P. Gerner-Schmidt)
- International epidemiological tools (D. Heyman)
- EU research support in emerging epidemics – current/future trends (L. Matthiessen)

Only a few genome sequencing options for the diagnosis of infectious diseases directly on clinical specimens without prior enrichment presently exist. However, this situation will most likely change dramatically in the near-future (5-10 years). The read length achieved by presently available technology is sufficient but sampling frequency is still too low, and the price of analysis likely is still an important impediment. In the future, for a system to be efficient, meta-genomic data for all organisms are needed.

There is a need to work towards global recognition of the total genome sequencing technique as the gold standard for microbial identification. The human metagenomic research project could serve as inspiration, but global expert consultations are needed in the pursuit of a global standard or nomenclature within WGST. Another first step could be the creation of a database of full sequences combined with basic user-friendly software tools for clinicians. For virology, sequence-based methods for diagnosis and typing are already widely used, but based on partial genome sequence data. There are lessons to be learned from this experience, for instance about standardization, feed-back modules on central databases, as well as more generic approaches to data sharing, - integration, - mining, and translation of findings for clinicians, epidemiologists, infection control staff and other stakeholders.

WGST has already been adopted as a supplement to current laboratory typing methods for virus research but is lacking in the field of bacteria. Up until today WGST has not saved time in outbreak investigations. However, the technology has a great potential in supporting timely and accurate outbreak investigation if all or a large fraction of pathogen isolates are sequenced in near real-time. This will of course require global collaboration between various scientific disciplines which will need to be supported by e.g. communication across disciplines, networking, stakeholder consultations etc.

When developing sequence databases, another important aspect is for the data to be curated. This could be conducted automatically; however, this would require several inbuilt features such as uploads of meta-data (time, location, host, source etc.) to be verified by the submitter, rejection of poor quality reads, and other alert functions. The task of curating the database should be maintained by supra-institutional organizations that already have extensive experience in the curation of genomic sequence data (e.g. NCBI, EMBL, etc.)

Building a global detection and control system based on next-generation technologies such as WGST calls for funding and mutual commitment. It will be essential to include stakeholders and political decision makers in this collaborative effort, which should also involve IP expert, industry experts, clinicians and in general people with expertise in epidemiology, bioinformatics, mathematical modeling, and other microbiological disciplines.

3. CONCLUSIONS

Points for a conceptual framework

Recent disease outbreaks have reinforced the notion that infectious diseases remain a global challenge. Fifteen million (>25%) of 57 million annual deaths worldwide are the direct result of infectious disease and it is generally agreed that current and new infectious disease challenges will continue to arise, given the trend towards globalization of travel and trade, coupled with demographic changes (urbanization, ageing) and increasing impact of the human population on natural environments.

The rapid development of genomic technologies and the potential replacement of century-old bacterial culture diagnostic technique in clinical laboratories holds great promise for improving the early detection, prevention and control of current and emerging infectious diseases, thus contributing to improved health of the global population. This can only be done, however, through effective collaboration across disciplines (e.g. clinicians, microbiologists, epidemiologists, bioinformaticians and others), while respecting legal and ethical rules and regulations.

This meeting discussed the potential development of a global system to aggregate, share, mine and use genomic data to direct part of the genomics efforts to address global public health and clinical challenges, a high impact area in need of focused effort.

The meeting concluded that such a system could be deployed in such a way as to promote equity in access and use of the current technology worldwide, enabling cost-effective improvements in health. Furthermore, developing such a system is both urgent and imperative, as technological progress and market forces will result in widespread deployment of genomic sequencing technology for routine diagnostic testing in clinical laboratories.

Meeting participants agreed to the following concluding statements:

A. CONCEPT

1. A global system or at least interoperable systems to aggregate, share, mine and translate genomic data for microorganisms in real-time is a realistic goal to achieve within a five to ten years horizon. A system enabling a direct link from end-users (e.g. clinician, veterinarian, clinical laboratorian, epidemiologist, academic researcher) to main databases through user-friendly platforms would provide significant advantages. This system could include a reference which could be accessed both for single clinical tasks (simple microbiological identification) as well as for national and international public health surveillance and outbreak investigation and response.
2. A pre-requisite for such new developments is a fundamental shift in the current paradigm which is mainly focused on single pathogens. Merging access to laboratory data across traditional discipline groupings (e.g. 'virology, bacteriology, parasitology' or 'animal, food, human') could potentially result in major cost-saving developments in the near future. It is likely that such changes will be easier in countries where systems are not yet historically consolidated, enabling significant 'leap-frog' (i.e. a technological short-cut) potential for developing countries.

3. System transparency and openness, along with precise data sharing rules (e.g. potential for downloads of data, consented scope for use of data), are very important aspects of any future system. Issues will arise in outbreak situations where some discretion and data restriction will be prudent, taking due care of the need to follow the WHO International Health Regulations (2005). Decisions on what metadata (e.g. demographic, geographical, sociological) or strain-related data (e.g. phenotypic, genotypic) to include need to be made as well as descriptions of the multiple potential ways of getting such data into the system(s). An analysis of the impact and feasibility of enforcing rules of immediate and unlimited posting of everything should be made.
4. A key component of the system would be translational activities to provide information to stakeholders and end-users, including clear interpretation of all outcomes. A clear description of the uses of the data is needed for acceptance and successful implementation, as well as a description of the organizational set-up responsible for the day-to-day running of and regular feed-back from the system. It should be noted that the main benefit is likely to be at the local level – the global epidemiological potential, including outbreak control, will be an additional outcome. Articulating explicitly the utility of any future system is important – providing added value is key, such as faster, cheaper or more accurate identification.

B. TECHNICAL SET-UP

5. A number of structural schemes could be envisaged for the system, including for example a global system involving data centers at the national or regional level with a direct link to major genome and sequence repository databases and evaluation systems.
6. Standards for and harmonization of data formats and technological systems involved are key to this development. It is to be anticipated that (multiple) standards will develop anyway – it is thus important to guide this standardization logically. This could be done under the auspices of the World Health Organization and the World Organization for Animal Health (OIE), but standards do not necessarily need to be developed in a top-down approach. Lessons could be drawn from the human microbiome standardization projects (<http://www.microbiome-standards.org/index.php?id=202>), and other projects (<http://ec.europa.eu/eahc/projects/database.html?prjn0=20084153>).
7. Interaction and cross compatibility with legacy systems will facilitate a smoother transition to a whole genome sequence typing (WGST) system. While the new system will provide greater information content, current systems are rich in data points exceeding 100,000 in some cases. Wide acceptance of this WGST platform will be accelerated if the new data can be placed into the context of previous and currently existing typing/detection systems. During a start-up period (years), where the database(s) are being created and data uploaded onto a database with existing genomic data (e.g. MLST) could provide a critical link to the future.
8. Upon uploading data, instant automated reports such as treatment guidelines, molecular typing information as well as spatial and temporal data will be a very important component to ensure the success of any such system. It will be important for a system to serve well the needs of its end users, including clinicians and veterinarians ordering diagnostic tests, public health officials engaged in disease surveillance and outbreak investigation and response, and basic and applied researchers. This will provide the win-win outcome that could promote this endeavor as a successful and globally worthwhile development – a global good. It is important to assess and address the needs of the end users during the establishment of the system, as part of a broader needs assessment. Furthermore, an easy-to-understand graphical interface will be key for use by both private and public health stakeholders. When costs of genome sequencing decline to a competitive level, a surge in countries turning to this technology will be inevitable.

C. IMPLEMENTATION

9. Non-technical issues surrounding the implementation of a system such as this are critical. International public and veterinary health diplomacy will be needed, and the inclusion of relevant and existing inter-governmental bodies needs to be ensured. Engaging stakeholders, relevant funding institutions and the private sector, as well as all technical constituencies will be important to this effort.

10. Communication of the importance and usefulness of a system such as this is a critical component to the success of the proposed way forward. This includes communication to the science level, public health level, political level, etc. To make a compelling case, examples that illustrate clear benefit for the individual patient, for public health and for economic growth need to be evaluated and presented. The system will need to provide easy to understand, plain language reports to the people at each level. Development of this system needs to be taken out of the academic sphere and into the public health sphere as well as the general political arena. A detailed road map for further next steps should be developed and agreed upon by all parties involved, from the scientific to the public health and political levels. The meeting organizers will work on developing a broad, inclusive list of participants in the road-map creation, along with “consultative” status entities.

11. There are several obstacles for the free sharing of genomic data. Important examples are: a) the reluctance of researchers to share data before a potential publication, b) the reluctance of governments and institutions sharing data, when weighing the need to protect personal identifying information and conduct effective outbreak investigations, and when competing interests are in play (e.g. trade, tourism etc.), c) legal and ethical issues, and d) confidentiality during public health emergencies. Acknowledging such constraints, promoting uploads of full genome data in a timely fashion could be assured in a number of ways. Specific changes in the rules applied by funding agencies could have a major impact, while the Genbank example shows the power of scientific journal policies to affect such developments (i.e. by requiring sequences to be deposited in GenBank before official publication), and other incentive schemes could be envisaged. Within this context it will be important that intellectual property rights issues be analyzed and understood in order to promote the free release of data. It is also important that sufficient emphasis be placed on the ability to use information immediately in case of public health emergencies.

Annex 1:

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Annex 2:

Agenda

01 Sep. Morning: Present Potential and Perspectives

- 09:00-09:25 Welcome and Vision + present initiatives (Schlundt, DK)
09:25-09:50 The Center for Genomic Epidemiology (Aarestrup, DK)
09:50-10:15 Data technology possibilities – database possibilities (Lund, DK)
10:15-10:40 The global landscape relative to gene sequencing (Gilmour, Can; Keim, USA)
11:00-12:00 Break-out Groups: *Is the vision realistic?*
12:00-12:30 Plenary discussion: *Is the vision realistic?*

01 Sep. Afternoon: Global policy context

- 14:00-14:25 International Health Regulations – the need for sharing (Lo-Fo-Wong, WHO)
14:25-14:50 International perspectives – laboratory links (Koopmans, NL)
14:50-15:15 CDC's Global Disease Detection Programme (Jeremy Sobel, USA)
15:15-15:40 Potential for innovative solutions in developing country settings (Keim, USA)
16:00-17:00 Break-out Groups: *Global need – Global commitment?*
17:00-17:30 Plenary discussion: *Global need – Global commitment?*

02 Sep. Morning: What to do – and how to do it?

- 08:30-08:55 Dealing with outbreaks – sequencing as an epi tool (Harmsen, D)
08:55-09:20 Future Laboratory Networking (Kroneman, NL)
09:20-09:45 A world without culture isolates (Gerner-Schmidt, USA)
09:45-10:10 International epidemiological tools (Heyman, UK)
10:10-10:35 EU research emerging epidemics – current/future trends (Matthiessen, EU)
10:50-11:40 Break-out Groups: *How to proceed?*
11:40-12:00 Plenary discussion: *How to proceed?*
12:00-13:00 **Plenary discussion: *Final Conclusions?***

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