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AD HOC GROUP ON HIGH THROUGHPUT SEQUENCING, BIOINFORMATICS AND COMPUTATIONAL GENOMICS (HTS-BCG)[1]

Paris, 25–27 November 2013

An *ad hoc* Group (AHG) on High Throughput Sequencing, Bioinformatics and Computational Genomics (HTS-BCG) was convened at the OIE Headquarters from 25 to 27 November 2013.

The Agenda and List of Participants are given at Appendices I and II, respectively.

1. Opening

Dr Elisabeth Erlacher-Vindel, Acting Head of the Scientific and Technical Department, welcomed the participants of the meeting on behalf of Dr Bernard Vallat, Director General of the OIE. She explained that the specific task of the Group was to develop an OIE strategy on the topic for use by the OIE, the OIE Reference Laboratory and Collaborating Centre (Reference Centre) network and the Member Countries (MCs).

2. Appointment of chairperson and rapporteur

The meeting was chaired by Prof. Massimo Palmarini, and Dr Peter Daniels was designated as rapporteur.

3. Terms of Reference for the *ad hoc* Group meeting

The Terms of Reference (ToRs) were adopted with minor modifications; they can be found at Appendix III.

4. Summary of key recommendations

4.1. The genome sequence of infectious agents and its subsequent analysis are integral components of disease investigation now and in the foreseeable future.

4.2. The strengths of the OIE include its animal disease information system and its network of 284 Reference Centres. Accordingly, the inclusion of the genomic sequence data of infectious agents should be an integral part of reports of animal disease and surveillance. Consequently, the OIE should adapt its animal disease information systems to include this information.

4.3 HTS-BCG will increasingly be a major tool in the generation of genomic sequence data, and the OIE should develop standards for the management of this technology in laboratories and the inclusion of HTS-BCG procedures in the laboratory methods for specific animal diseases. These matters should be urgently progressed through the development of pilot projects that will address the database issues, develop the standards for inclusion in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*), initiate networking among suitably qualified Reference Centres and produce standard data sets for use in quality assurance programme.

[1] Note: This ad hoc Group report reflects the views of its members and may not necessarily reflect the views of the OIE. This report should be read in conjunction with the February 2014 report of the Biological Standards Commission because this report provides its considerations and comments. It is available at: http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/laboratories-commission-reports/meetings-reports/

5. Addressing the Terms of Reference

5.1. ToR 1: Globalisation of Microbial Diagnostics in Animal Health

The AHG noted the increasing role that sequence information is playing in the diagnosis and management of microbial infections, including in the characterisation of infectious agents, their possible phenotypic characteristics and the likely distribution of their spread from place to place and through time. The AHG recommended that the OIE should develop a strategy, and adopt policies and practices on analysing and managing genomic sequence data of microorganisms as they are becoming essential to the understanding and control of infectious diseases.

The AHG considers that in the current and future scientific environment a microorganism is not identified satisfactorily unless essential features of its genome are described. For viruses this may be the whole genome while for bacteria and parasites, it may be just partial sequences. However, the technology is growing so rapidly that within a short time whole genome sequences for these larger microorganisms may also be routinely generated.

The OIE has the responsibility to be the leading agency internationally on matters relating to animal health. Consequently, for the OIE to discharge this mandate, it will be necessary to play a leading and central role in the management, interpretation and use of sequence information in matters related to animal health. This will include but not be limited to the development of standards for the generation of such data during investigations of infections of single animals, animal populations and their immediate environment and at any point along the "value chain" linking animal production with the ultimate beneficiaries.

The AHG noted that the clear trend to increasing reliance on generating and using sequence information for the study of infectious agents has implications not only for the generation, management and use of such data, and the need for accompanying standards and services, but also ultimately for the design and management of veterinary laboratories. This might ultimately include devices and systems for the generation of sequence data away from the laboratory and closer to the point of sampling on the farm or at other points along the "value chain" including animal health and food safety. Such developments will require standards to ensure supervision by the veterinary services and adequate quality assurance.

5.2. ToR 2: A Global Network of OIE Reference Centres for a Coordinated International Approach to Implementation of HTS-BCG

The OIE through its designated Reference Centres and access to other national expertise through the offices of the official Delegates from MCs has at its disposal much of the expertise that would underpin the scientific understanding of the use of sequence information of microorganisms in relation to animal health. The AHG recommends that this expertise be harnessed to assist the OIE in further developing its policies and practices relating to managing and using sequence information in the discharge of its mandate.

In the first instance this should be progressed by involving suitable qualified scientists from the existing global network of Reference Centres to address specific matters on which advice will be needed from time to time. A more detailed approach to assessing the depth and location of such expertise is suggested in the AHGs report in Section 5.5 below.

5.3. ToR 3. Coordination of Data Management, and a Role for the OIE in Managing a Dedicated Database

The AHG further recommends that the OIE develops a more comprehensive approach to the collection and storage of sequence information relating to animal disease, and to the making of such information open access.

The AHG considers that sequence data of microorganisms such as generated by HTS, metagenomics approaches is only a tool, although a powerful one, to investigate issues regarding animal health and food safety. Consequently, the whole process of interpretation of sequence data in relation to the disease investigation should be led by suitably qualified veterinarians, consistent with the usual requirements for diagnosis of animal disease. To further emphasise that considerations of the sequence analysis will be an integral aspect of animal health decision making in the future, the AHG further recommends that the sequence and sequence analysis of infections associated with cases, outbreaks and investigations of animal disease should be recorded together with all other information relating to the reporting and recording of such cases and outbreaks, and be considered a necessary part of such reports and records.

Consequently, the AHG further recommends that the OIE require reporting of genomic information in formal reports to the OIE, and that it develops systems for the receipt, storage, accessing and sharing of such information.

The meeting was joined by Dr Paola Caceres and Dr Lina Awada from the OIE Animal Health Information Department, to assist the AHG to further explore practicalities and strategies for the implementation of this concept.

It is the understanding of the AHG that the storage of sequence information of microorganisms relevant to animal health is currently somewhat fragmented. Although GenBank is thought to be the primary repository of such information, the AHG is aware of other activities such as agent-specific databases maintained by some Reference Centres and a mixture of public and private databases for other agents such as influenza viruses. Importantly, it is common practice for public funding agencies to have a mandatory requirement for timely depositing of sequences generated by their funding on open access databases. The AHG is also aware of international discussions initiated with the purpose of consolidating information relating to microorganisms and accompanying metadata including epidemiological information.

Against this background, and recognising its complexity, the AHG discussed the role of the OIE and the stability it offers. The OIE is an organisation of voluntary membership of most of the countries engaged in animal health and production on the planet. OIE membership is formal, on the basis of being an official government membership. Furthermore it has been formally ratified internationally that the OIE shall be the official arbiter on matters and standards relating to animal health in international processes such as the WTO. Hence, the AHG suggests that it is appropriate, even necessary, for the OIE to take a leading role in the management of sequence information relating to infections of animals internationally. The OIE offers a stable and enduring base for such involvement.

The OIE already maintains a database of reports of animal disease situations from MCs. Consistent with above recommendations and considerations, the AHG recommends that the inclusion of sequence information be required in such reports, and that the OIE considers the technical requirements to expand its reporting and recording role in this manner.

In support of this recommendation, the AHG notes that the OIE has an established reporting framework supported by the legal authority and obligation, it is further supported by a laboratory network through which it might be expected that much of the relevant sequence information will be generated.

A possible negative consideration, to be further addressed by the OIE as it develops a business plan for uptake of this recommendation, is that of duplication among databases and their roles. A strategy to manage perceptions and real overlaps in this area would be necessary. The costs and resourcing implications of establishing a sequence database and managing it would also have to be addressed. For an OIE sequence database to be considered useful by the international scientific community it would have to be easy to use, operate effectively and managed efficiently.

It is the preference of the AHG to leave more detailed technical considerations to others with specific expertise, but note there was discussion within this Group on the strategies around linking the current OIE database, WAHIS, to other databases rather than being the primary repository of data. The AHG tended to consider that a strategy of just being a gateway linking to data elsewhere may not be consistent with the recommendation that the OIE develops its operations to continue to be the primary reliable source of animal health information globally.

5.4. ToR 4: Aspects of HTS Test Systems for which Standards should be Developed

5.4.1. The Range of Purposes for HTS-BCG

HTS-BCG is a powerful and versatile technology that can be deployed for a range of purposes in the detection of infectious agents and their characterisation, either in biological material such as diagnostic or surveillance specimens or propagated in cultures or as isolates. As such the users of the technology should consider the purposes of their testing in relation to the normal purposes of testing as defined in Chapter 1.1.5 *Principles and methods of validation of diagnostic assays for infectious diseases* of the OIE *Terrestrial Manual*.

Further to these general purposes of testing, HTS-BCG offers specific opportunities in:

- Detection, identification and characterisation of previously unidentified microorganisms;
- Improved diagnosis of known diseases;
- Improved diagnosis of emerging or re-emerging diseases with known or unknown aetiology;
- Single "universal" diagnostic assays, able to identify any potential pathogen, that can be developed in concert with established diagnostic approaches;
- Multiple agents that can be simultaneously and quickly detected in diseases with multifactorial aetiologies;
- Increased capability to study the evolutionary dynamics of pathogens at the farm, local, national and global level;
- Deeper understanding of the epidemiology of infectious diseases and the phylogeography of infectious agents;
- Enhanced traceability of infectious diseases and modes of pathogen transmission including applications in forensic epidemiology;
- More extensive characterisation of "populations" of known pathogens (e.g. relevant minority strains, escape mutants) that in turn facilitates the design of better vaccines, antivirals, etc.;
- Better links between pathogen genotype and phenotypes enabled through full genome sequence of multiple strains (including reference strains) of a single agent.

5.4.2. Sampling, Specimens, and Sample Preparation

HTS-BCG is a new technological tool in the management of diseases of animals and its use should be adopted within the context of tried and accepted processes for the management of animal health. In laboratories where it is utilised, it should be managed within the context of the habitual veterinary investigation process and within the context of the laboratory's quality assurance system. The use of any capability of the technology should be appropriate to the purpose of the investigation, and the sampling strategy and the specimens taken should be appropriate for that investigation, based on an understanding of the pathogenesis and epidemiology of the infection under study or the likely pathogenesis and epidemiology of any novel infectious agent suspected. Such investigations should be under the supervision of appropriately qualified veterinarians.

Similarly the results of HTS-BCG must be interpreted in the context of the pathogenesis and epidemiology of the infection in the animal species under study. Results should be reported by appropriately qualified veterinary investigators with the authority to make diagnoses of animal diseases under the laboratory's quality assurance system and in the jurisdiction where the investigation is conducted.

Specimens will be collected and submitted to the testing laboratory in accordance with the standards communicated in Chapter 1.1.1 *Collection, submission and storage of diagnostic specimens* of the OIE *Terrestrial Manual* and the normal comprehensive information regarding the individual animal, the case or reason for sampling and the relevant epidemiological information recorded in the laboratory's accessions process as for any submission to the laboratory.

As with other laboratory processes, and molecular techniques in particular, ensuring the integrity of the specimen and the samples to be tested is critical. HTS-BCG can be subject to contamination of samples during the processes of sample preparation and initial workup. Separation of work areas from the possibility of cross contamination with nucleic acid from other molecular investigations is an essential requirement.

5.4.3. Commercially Available Sequencing Platforms

There are a number of commercially available sequencing platforms or services for the purpose of generating sequence information from test samples. The choice of platform should be based on a consideration of the intended purpose or combination of purposes as outlined in Section 5.4.1 above.

Of primary concern is that the technology to be selected is fit for the intended purpose, that it is appropriate to produce sequence information from the types of genome intended for study. Other considerations may include the time required to conduct a sequencing run, including sample preparation; ancillary equipment needed in addition to the actual sequencing device; the capital cost of the purchase and set up of all necessary equipment and the cost of annual licences or service agreements; the availability of supporting expertise from the supplier; the cost of reagents for a sequencing run and the likely availability of reagents in the country concerned; the staff requirements to operate the equipment and to conduct the associated bioinformatic analyses and the data management requirements. Currently available systems have been reviewed (Belák *et al.*, 2013, OIE *Sci. Tech. Rev*.) but new models and technologies can be expected to frequently become available.

5.4.4. Bioinformatics

An absolute requirement for any laboratory intending to establish an HTS-BCG capability is the employment of specialised bioinformatics skills. In the future, academic or commercial suppliers may market platforms with supporting software for specific analyses in defined clinical situations, but the use of such packages does not remove the responsibility of the laboratory to be able to competently analyse its own data, and reliance on any such inbuilt analytic capability would seriously limit the potential of the technology for broader applications.

The bioinformatics that assemble the genomic sequence from the raw data and the subsequent analysis are the critical elements in HTS-BCG. Hence the approaches used must be transparent and a declaration of the software packages used should be a component of every report of sequence analysis. Software programs used for these analyses must be available (commercially or open access) in order to be evaluated by the international community.

As with any laboratory procedure, attention must be given to quality assurance. It is required that every sequencing run will include positive and negative controls appropriate to the investigation and that have been incorporated through the sample preparation processes of the sequencing run as well as the actual run on the technology platform. The test method should include criteria for acceptance or rejection of each run based on the satisfactory analyses of the controls.

The appropriateness of chosen bioinformatics software for particular analyses can be evaluated through testing its performance against standard data sets containing data relating to agents expected to be present in the specimens to be tested (see Section 5.5. below).

5.4.5. Data Management

The data generated from HTS-BCG operations are essential to reach the diagnosis or other scientific purpose of the investigation, and so are an integral component of the process. As such it is an essential requirement of laboratories to have policies, processes and supporting systems to curate, manage and store the data generated.

Different HTS technology platforms produce raw data in different formats and stages of pre-analysis so it is necessary for laboratories to have policies and processes specific for the technology platform in use. Data management systems will include aspects of which data to keep, and the length of time for which it will be kept, and the back-up strategies to protect against accidental loss or deliberate erasure.

Where a sequence analysis leads to an output of animal health significance, especially one of trade or international significance, it is an absolute requirement that the data on which the analysis was performed be kept available for audit or confirmatory analysis for a period of time commensurate with the significance of the animal health finding. This is particularly important where the finding may be disputed. Failure to be able to produce the required data for independent analysis could be taken to invalidate the finding.

Sequence data should be stored in a manner in which there is a clear link to the metadata associated with the specimen that was the subject of the analysis. As for other laboratory investigations, such metadata include information regarding the animal sampled, its ownership and location, and accompanying clinical and epidemiological information in the animal population.

5.4.6. Validation of Test Systems for Designated Purposes

The concepts of test validation as stated in Chapter 1.1.5 of the OIE *Terrestrial Manual* are broadly applicable to HTS-BCG. Stage 1 validation data must be developed to confirm the analytic sensitivity and specificity of the technique, and its repeatability. It is recognised that it may not be practical to produce large data sets on test performance such as would normally allow calculation of test diagnostic sensitivity and specificity, but other aspects of validation such as demonstration of test reproducibility among laboratories conducting similar investigations should be undertaken. Where proficiency testing strategies have been developed laboratories using HTS-BCG should participate.

5.5. ToR 5: Training, quality management and dissemination of knowledge

The AHG considered that a useful starting point would be a stock take of the level of use and expertise currently existing in the OIE Network of Reference Laboratories and Collaborating Centres (the Reference Centres). Hence it was recommended that the OIE, through the OIE Biological Standards Commission (BSC), should solicit information from Reference Centres by means of a questionnaire. Information to be sought should include:

- Whether laboratories currently utilise HTS-BCG
- Whether laboratories maintain their own sequencing capability, or outsource the sequencing
- Whether laboratories maintain in-house bioinformatics capability, or outsource the bioinformatics analyses
- For what disease investigations or other purposes do they utilise HTS-BCG
- A list publications arising from their work or collaborations with others

Such a survey of the Reference Centres would indicate the base of capability in the OIE network, facilitate identification of centres of expertise for involvement in network activities and help to spread awareness of the level of OIE interest in this technology.

Regarding training, it was noted that this would most usefully be conducted within the context of accepted OIE standards for HTS-BCG, the foundations for which have been outlined in Section 4.4 above.

Regarding quality assurance it was noted that standard data sets against which the usefulness of bioinformatics software packages could be verified would be useful. The AHG recommended that selected Reference Centres with relevant expertise and data resources should be brought together to develop this concept and use their combined resources to construct standard data sets. It was further recommended that these data sets be recognised as OIE Standards and be maintained by the OIE for MC access.

Once a user network has been identified and HTS-BCG standards agreed there will be a role for proficiency testing (PT). An appropriately qualified Reference Centre could be approached to coordinate network Reference Centres with relevant expertise and access to resources of appropriate biological materials to develop a PT strategy relevant to one or more purposes of HTS-BCG testing and construct PT panels accordingly.

Regarding dissemination of knowledge the opportunity of the scheduled meeting of OIE Reference Centres in October 2014 was noted. The AHG recommended that the programme include information sessions on HTS-BCG and the status of the draft OIE Standards relating to that technology, the recommendations for inclusion of test methods in the OIE *Terrestrial Manual*, where appropriate, and the activities of the OIE HTS-BCG network of Reference Centres.

6. Conclusions

The AHG recommends that rapid progress be made on matters covered in this report. This could be addressed by pilot projects regarding the database requirements, the development of standard data sets, and the questionnaire to Reference Centres. The BSC should consider development of the standards recommended in this report as a draft chapter for the OIE *Terrestrial Manual*.

7. Finalisation and adoption of the draft report

The AHG finalised and adopted the draft report.

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Appendix I

AD HOC BRAINSTORMING GROUP ON HIGH THROUGHPUT SEQUENCING, BIOINFORMATICS AND COMPUTATIONAL GENOMICS (HTS-BCG)

Paris (OIE Headquarters), 25–27 November 2013

Agenda

1. Opening
2. Appointment of chairperson and rapporteur
3. Terms of Reference for the *ad hoc* Group meeting
4. Summary of key recommendations
5. Addressing the Terms of Reference
6. Conclusions
7. Finalisation and adoption of the draft report

Appendix II

AD HOC BRAINSTORMING GROUP ON HIGH THROUGHPUT SEQUENCING, BIOINFORMATICS AND COMPUTATIONAL GENOMICS (HTS-BCG)

Paris (OIE Headquarters), 25–27 November 2013

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Appendix III

AD HOC GROUP ON HIGH THROUGHPUT SEQUENCING, BIOINFORMATICS AND COMPUTATIONAL GENOMICS (HTS-BCG)

Paris (OIE Headquarters), 25–27 November 2013

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Terms of Reference

Develop an OIE strategy in the context of:

1. The globalisation of microbial diagnostics in animal health.

2. The possibility of establishing a global network of OIE Reference Centres for a Coordinated International Approach to implementation of HTS-BCG.

3. The coordination of data management, in particular the possible role for the OIE in managing a dedicated database.

4. The need to develop standards for inclusion in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*) on the following aspects of HTS test systems:

    4.1. The range and purposes for HTS-BCG

    4.2. Sampling, specimens and sample preparation

    4.3. Commercially Available Sequencing Platforms

    4.4. Bioinformatics

    4.5. Data management

    4.6. Validation of test systems for designated purposes.

5. Training, quality management and dissemination of knowledge on the use of these new tool bags.

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