A report to the Director-General of WHO

The Independent Advisory Group on Public Health Implications of Synthetic Biology Technology Related to Smallpox

Geneva, Switzerland

29-30 June 2015

WHO/HSE/PED/2015.1

Contents

Executive summary

Purpose

The purpose of the meeting was to provide guidance to the WHO Director-General on the public health implications of synthetic biology technology as it relates to public health measures for smallpox preparedness and control. The public health implications are for countries and for WHO, and include diagnostics, vaccines and medicines and research related to the variola virus.

Background

At the Sixty-seventh World Health Assembly in May 2014, WHO was requested to undertake a consultation on the use and potential impact of technologies for synthetic biology on smallpox preparedness and control, in order to further inform the World Health Assembly in its discussions on the timing of the destruction of existing variola virus stocks. As part of this consultative process a group of experts, the Independent Advisory Group (IAG) on Public Health Implications of Synthetic Biology Technology Related to Smallpox, was convened at the end of June 2015 in order to provide an assessment to the Director-General.

Prior to the meeting of the IAG, a Scientific Working Group (SWG) on Synthetic Biology and Variola Virus and Smallpox was convened on 16–17 April 2015 to review the evidence and inform the deliberations of the IAG. The SWG addressed relevant scientific and technical questions around the re-creation of variola virus; the risks and benefits of synthetic biology for variola virus research; and the guidance required from WHO regarding distribution and handling of live variola virus maintained at the two designated WHO collaborating centres that are authorized repositories of variola virus. The conclusions of the SWG were as follows:

> *"With the rapid advances in synthetic biology, there is now the capability to recreate the variola virus, the causative agent of smallpox. While recreating variola is quite complex, it is increasingly possible due to the availability of genetic material and of machines for complex assembly, as well as increasing know-how among a broad array of persons. Furthermore, the rapid rise in availability of genetic material from commercial sources and the so-called "grey market" is driving the cost of this material down, making recreation possible by multiple institutions and persons, including those with malicious intent. The "WHO Recommendations concerning the distribution, handling and synthesis of variola virus DNA" should be revised. Consideration should be given to adding a component or separate document on guidance to commercial DNA providers for screening requests for DNA fragments.*
>
> *With the development of these technologies, public health agencies have to be aware that henceforth there will always be the potential to recreate variola virus, and therefore the risk of smallpox re-emerging can never be fully eradicated."*

Methodology

A scenario approach was chosen to foster discussion and debate on the implications of synthetic biology among the members of the IAG. They were expected neither to comment on the likelihood of occurrence of the scenarios nor to provide solutions or recommendations for them; rather the aim was to have illustrative events to frame and guide the discussions.

The four scenarios described the following situations and were followed by discussions on the question of the impact of synthetic biology on controlling the emergence of smallpox disease.

- Scenario 1: the emergence of smallpox-like disease in a remote area in a developing country.
- Scenario 2: the emergence of smallpox-like disease in a densely populated city.
- Scenario 3: the emergence of smallpox-like disease following a laboratory accident.
- Scenario 4: a situation in which a group of individuals inject themselves with synthesized variola virus.

Implications

Before synthetic biology was possible, two potential situations for re-emergence existed: (1) natural re-emergence (e.g. by mutation of another poxvirus), and/or (2) a laboratory release (deliberate or accidental) from one of the two WHO variola virus repositories or from unknown locations.

Therefore, the risk of re-emergence is not new. However, it has increased as synthetic biology technologies to recreate, and even modify, the virus continue to become cheaper and more easily accessible. Even if the live virus stocks are destroyed, the act of destruction is not irrevocable.

Given that the risk has changed, the key questions facing the world now are:

- Is the world prepared for this additional risk? If not, what should be done to become sufficiently prepared?
- What are the implications for (1) public health preparedness for re-emergence and (2) research around this new risk?
- Does the new, additional risk change the parameters of the discussion for the destruction of the variola virus in the two repositories?

Based on recent reviews of the public health responses to the H1N1 pandemic and to the Ebola outbreak, there is recognition of fundamental gaps in several areas of preparedness for any emerging disease outbreak, including smallpox. Risk reduction strategies and preparedness need to be adapted to take into account this new risk from synthetic biology in addition to the core capacities already required under the International Health Regulations 2005 (IHR 2005), including:

- **Increased capacity for early detection and diagnostics:** clinical capacity to recognize and treat smallpox; laboratory capacity, particularly at local level; development of simple diagnostic tests.
- **Increased capacity for disease control:** anticipation and preparedness of requirements for public health countermeasures such as vaccines and antivirals; supplies and quantities of vaccines and drugs needed for different scenarios; global expertise in specific epidemic diseases such as smallpox.
- **Increased biosecurity:** revised regulations for research on DNA fragments of variola virus and synthesis of variola virus DNA by new technologic approaches;; increased biosafety and biosecurity in laboratories, including stock inventories; strengthened regulatory frameworks and their implementation; coordination between sectors including health, judiciary, law enforcement and customs.
- **More effective risk communication:** acknowledgement of risk in the context of other infectious risks; transparency regarding dangers and measures to prevent and avoid them; avoidance of attempts to mislead the public; tracking of and responding to rumours; community engagement to detect and manage disease and to assist in information sharing.

Synthetic biology goes beyond smallpox and should be considered in relation to the elimination and eradication of other infectious diseases as well.

1. Background

1.1 Objectives and process of the consultation

The Independent Advisory Group (IAG) on Public Health Implications of Synthetic Biology Technology Related to Smallpox met at WHO headquarters on 29–30 June 2015. The purpose of the meeting was to provide guidance to WHO's Director-General on the public health implications of synthetic biology technology, as related to public health smallpox preparedness and control. The implications reviewed were for countries and for WHO, including diagnostics, vaccines and medicines and research related to the variola virus. The agenda of the meeting is contained in Annex 1. A list of the members of the Independent Advisory Group and of the resource persons who attended the meeting is contained in Annex 2.

The meeting was opened by Dr Keiji Fukuda who welcomed the participants. Dr Fukuda stressed the importance of the meeting, noting that the matters to be discussed would be significant not only for smallpox but also for other issues. Although the disease smallpox had been eradicated, Dr Fukuda noted that the virus still exists in research laboratories. In addition, with the development of synthetic biology the recreation of variola virus has become a possibility. He pointed out that the issue of the timing of the destruction of the variola virus is a matter for discussion by WHO Member States.

The topic was being addressed because the World Health Assembly in 2014 had asked about the significance of synthetic virology in relation to smallpox. The Director-General would respond to the Health Assembly's question and the meeting of the Independent Advisory Group would be a major part of preparing that response. The Director-General would consider the advice given by the IAG and would decide how to disseminate the report.

The meeting was attended by members of the IAG as well as four internationally renowned experts on variola virus and smallpox. All participants were asked to treat the discussions as confidential.

1.2 Overview of smallpox

Dr Sylvie Briand gave an introduction to smallpox disease which has been a cause of fear for centuries and for which the first ever vaccine was developed in 1796. WHO's smallpox eradication programme was started in 1966, and the last natural case of the disease was reported in 1977 in Somalia. The World Health Assembly declared smallpox eradicated in 1980. However, the variola virus has not been eradicated, as it was decided at that time to maintain stocks in the Soviet Union and the United States. The live virus is today maintained at two WHO Collaborating Centres: the Centres for Disease Control and Prevention, Atlanta, USA (CDC), and the State Research Centre of Virology and Biotechnology, Novosibirsk, Russian Federation (VECTOR).

Humans were the only reservoir for the disease. The disease course has a long incubation period of 7–17 days. People are infectious only from the onset of rash and remain infectious until disappearance of the last scab. Transmission is through large and small particle aerosols, and direct contact with body fluids. The disease is also spread by fomites. In its last decades the disease was chiefly spread in households and health-care settings. In a susceptible population the average number of cases of smallpox generated by an infected person is 3–6 (while for measles it is 12–18).

The symptoms of the disease can be confused with other diseases such as monkeypox and even chickenpox. Various vaccines and diagnostics are available, and antivirals are being developed. In addition there is VIG (Vaccinia Immune Globulin) which is made from the blood of individuals who have been vaccinated with the smallpox vaccine. Given the research of recent decades there are more interventions available now than there were in the pre-eradication period.

During the Smallpox Eradication Programme, the vaccine used had serious adverse reactions (5-10 in one million vaccinees), and 1-2 in one million died. However, vaccination was carried out because the risk from the vaccine was less than the risk from the disease. Today the vaccine could not be used in certain populations, such as persons with HIV. Although attenuated, safer vaccines are available, there is uncertainty about their effectiveness and safety in mass campaigns.

It is highly likely that an outbreak of smallpox will lead to similar challenges to those posed by the recent Ebola outbreak in West Africa or MERS-CoV outbreak in the Republic of Korea. Major priorities in such an outbreak would be fear management, risk communication and community engagement to promote compliance with control measures and reduction of stigma. If several countries are infected there will need to be rapid delivery of vaccines, and countries would be expected to want more than they need. The global emergency stockpile of smallpox vaccine is 2.4 million doses in Switzerland, plus 32 million doses donated and available in other countries. The overall quantity of vaccine available is estimated to be around 700 million doses. There is a lack of clarity about the length of immunity after vaccination, and the only certain lifetime protection comes from having already had smallpox once.

The main concern in an outbreak of smallpox would be that no one under 40 years of age (around 3.6 billion people) has been vaccinated, thus leading to an increasingly susceptible population. There would be delays in diagnosis since the disease is unknown by many health workers, and a global panic can be anticipated even with only a small number of cases.

There would be a major economic impact – the 2015 outbreak of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in the Republic of Korea is estimated to have cost the Korean economy many billions of dollars. .

Meeting participants noted that little is known about potential hidden stocks of variola viruses. At the same time, the development of synthetic biology reduces our confidence in eradication. If the disease were to reappear, whether naturally or due to a synthetic virus, we would have 14 days (maximum incubation period) to vaccinate contacts. However, given the intensity of international travel, the disease would potentially spread rapidly and, since health systems in developing countries are often under-resourced, delays in diagnostics can be anticipated.

1.3 Report of the Scientific Working Group

In April 2015 WHO convened a meeting of a Scientific Working Group (SWG) on Synthetic Biology and Variola Virus and Smallpox. The report of the SWG, which was presented to the Independent Advisory Group by Professor Geoffrey Smith, is attached as Annex 3. The aim of the SWG was to provide scientific background information on synthetic biology technology with regard to the variola virus.

The SWG concluded that, with the increasing availability of DNA fragments that can be synthesized from simple chemicals, it would be possible to recreate variola virus, and that this could be done by a skilled laboratory technician or by undergraduate students working with viruses in a relatively simple laboratory. To recreate the variola virus by synthesizing the DNA and then relinking the parts is theoretically possible but is considered to be highly unlikely to happen accidentally since the variola genome is large and complex. It was therefore felt that synthesis would require a deliberate and sustained effort.

Over 45 genomes of the variola virus have been sequenced and the sequence is in the public domain, and other orthopox viruses (which have similar DNA) have been recreated. Recreating variola virus is prohibited by under the World Health Assembly (WHA) resolutions, though anyone trying to do this may not know or care about WHO's rules. Someone recreating the virus could, either by accident or deliberately, introduce elements to enhance its virulence or make it resistant to existing medicines and vaccines.

The SWG noted that, although a recreated variola virus could be useful for research on vaccines and diagnostic tests, there are serious concerns regarding modifications of the virus by institutions or individuals with malicious intent. Members of the SWG concluded that the WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA should be revised. Additionally, consideration should be given to the addition of a component or separate document on guidance to commercial DNA providers regarding screening of requests for DNA fragments. The SWG further concluded that, given the ability to recreate the variola virus using synthetic biology techniques, the destruction of the remaining stocks of variola virus at the two WHO Collaborating Centres would not irrevocably destroy the virus. After destruction of the stocks, the variola virus could still be recreated.

2. Independent Advisory Group discussions

2.1 Methodology of scenario-based discussions

A scenario approach was used to foster discussion among the members of the IAG on the impact of synthetic biology on smallpox preparedness and control. Participants were asked to discuss what would be the implications of synthetic biology in the following fictional scenarios, where the re-emergence of smallpox would be suspected.

- Scenario 1 described the emergence of smallpox-like disease in a remote area in a developing country.
- Scenario 2 described the emergence of smallpox-like disease in a densely populated city.
- Scenario 3 described the emergence of smallpox-like disease following a laboratory accident.
- Scenario 4 described a situation in which a group of individuals inject themselves with synthesized variola virus.

The purpose of discussions was not to comment on the likelihood of each scenario happening, but rather to focus on what would be likely to happen if such a situation occurred and the role of synthetic biology. The ensuing discussion for each scenario is summarized in the next section.

2.2 Discussion of the scenarios

2.2.1 Scenario 1: A remote area in a developing country

Summary

- Challenges in recognizing the clinical symptoms of the disease.
- Challenges in laboratory diagnosis and confirmation (at least 8 weeks) requiring a reference laboratory.
- Limited use of synthetic biology for the rapid development of control measures.

Members of the IAG agreed that the emergence of smallpox in a remote area in a developing country raised a number of specific issues related to the poor state of the health system and the remoteness of the event. Remoteness meant that the virus was unlikely to spread as rapidly as in an urban environment, and could probably be contained in the area without international spread. However, it would also delay the arrival of outbreak investigators to the area and, once there, they may not suspect smallpox. Local laboratories would have no possibility to diagnose the disease. A national reference laboratory would find it was a poxvirus but this was likely to mislead the authorities into thinking it was monkeypox or another poxvirus. Monkeypox is endemic in the Democratic Republic of the Congo and appears to be increasing; it probably also exists in other tropical countries. Only after a WHO collaborating centre examined samples would it be possible to identify smallpox, by which time almost eight weeks would have passed.

Possible solutions in this scenario could include mobile telephone apps that recognize the nature of disease from a photograph, or dipstick-like diagnostics. The latter are already being developed by CDC to be orthopox-sensitive and variola-specific.

It was felt that most public health laboratories in Africa would be able to identify a virus as an orthopox, but a reference laboratory would be required to identify variola.

It would be beneficial to have a reference standard against which to measure a circulating virus; thus, if there was no live variola virus in the repositories, a new one could be created as a reference using synthetic biology. However, this would take months or years with the current technologies. One could use only the new isolate, which would be quicker, but this would not provide the same level of certainty.

A possible solution discussed by the IAG would be to supply the live variola virus to local or regional reference laboratories to help in diagnosis. However, it was acknowledged that this requires consideration of the risk of unintentional or intentional release of the virus relative to required biosafety and biosecurity measures for a virus which has been kept under tight security since 1980.

The general opinion was that synthetic biology has had little impact on the work of the collaborating centres on variola virus. Synthetic biology has been used partly to develop some of the proteins on the surface of the virus. If a new strain of variola virus with different characteristic than the current live variola virus were developed and released, it is highly unlikely that synthetic biology could be used to develop an effective vaccine quickly.

2.2.2 Scenario 2: A densely populated city

Summary

- Transmission will be faster in densely populated areas and contact tracing will be extremely difficult.
- Rapid scale-up of laboratory diagnostic capacity will be required in many places in the case of re-emergence.

The IAG again raised the issue of the capability of the health system to detect the virus, coupled again with delays in identification due to a complete lack of familiarity with it. After all, no one looks for an illness that has been eradicated. The crowded environment in a city increases the chance of spread, and the fact that patients can easily visit several doctors and hospitals also increases the risk of further infections. Contract tracing would be a major undertaking. While persons with infectious smallpox are usually so sick that they will not travel, the fact that the cities are usually close to ports and/or airports, means that the chance of spread to other areas and countries is enhanced.

While this scenario had a shorter timeline from detection to diagnosis than the first one, the scale of potential infection is also much greater. Some members felt, however, that making the diagnosis of smallpox within 20 days would be very optimistic, as the likelihood would be that a zoonotic illness would be suspected and searching for this would take time. On the issue of diagnosis, the view was expressed that, if we know we are not able to eliminate the risk of smallpox definitely, we should not destroy the tools and experience we have, and hands-on experience will be very important, at least for the next 30 years. However, a contrary view was that there will continue to be a great deal of genetic research in the coming years and much of this will apply to variola virus, so expertise in this area is likely to increase rather than diminish.

Asked how viable it would be for the collaborating centres to supply primers to laboratories elsewhere at the request of WHO, VECTOR said it would send the primers and assay kits within a week. CDC noted it would take longer due to approvals but they would do it, and they would want to do proficiency testing to ensure that the primers work in a different environment.

2.2.3 Scenario 3: A laboratory accident

Summary

- A laboratory accident is always possible but the risk can be minimized by training of laboratory staff and strict application of biosecurity norms.
- The risk of accident is higher in illicit and unregulated laboratories.

In this scenario of a laboratory accident, it was noted that a national microbiological institute should not possess variola virus. In a recent case (US National Institutes of Health (NIH), USA 2014), where unlabelled vials of variola virus were discovered, the contents were presumably thought to be old, but safe, material. In that situation it was fortunate that the person who handled the vials did so carefully and according to training, and appropriate biosafety procedures were followed upon discovery. In the USA the discovery of these old variola vials led to NIH, CDC and others carrying out a full stocktake and catalogue of all stored materials.

Members of the IAG said the scenario showed the need for training and strict compliance with procedures to avoid such an accident. However, it was felt that this kind of accident is always possible – not only in amateur laboratories but even in high-level ones. Procedures would probably be much less strict in a laboratory doing illicit research with variola virus

Some members felt that in the first three scenarios it was astonishing that someone would ever consider that the cause might be a poxvirus. In reality, it would generally take a very long time to come to that conclusion. It was also noted that to distinguish a natural virus from a newly recreated one would require genome sequence determination and comparison, and if these were identical, the viruses could not be distinguished.

Asked the question whether, if all the current samples were destroyed, they could be recreated after destruction of the originals, both CDC and VECTOR replied that WHO recommendations prohibit them from doing so. Not all samples have been sequenced so a number of them could not be recreated anyway, though CDC reported it had been asked to sequence all samples and had partly done so. Professor Smith said there was no need to work with the live variola virus in order to be able to respond to a future possible smallpox outbreak. One could use other orthopox viruses to maintain this skill set in a trained staff under containment conditions.

2.2.4 Scenario 4: Individuals inject themselves with synthesized variola virus

Summary

- Re-emergence of smallpox will create fear and panic which can have an impact far worse than that of the disease itself.
- Rapid confirmation of suspected cases will be essential; thus the rapid increase of diagnostic capacity will be critical.

In this scenario, the IAG felt that the major issue to be faced was widespread fear and panic. Services would soon be overwhelmed and emergency stocks of vaccines would be insufficient.

Advisory Group members were agreed that this was a nightmare scenario that would go beyond the health sector and would be an issue of the highest order in every country. There would be insufficient vaccines, test kits, capacity and everything else, and the widespread public panic would be complicated by politics. Communication is important, but the entire system of communication would likely be disrupted. Rumours would spread on social media and the authorities would spend their time responding to problems rather than anticipating and preparing for them.

As for research and diagnostic capacity, CDC has five persons working on live variola virus and some others who are trained to do so; through the various US services, diagnostic capacity could be scaled up fairly quickly. However, in this scenario, it could be possible, if WHO so decided and Member States were in agreement, to downgrade the pathogen to Biosecurity Level 3 so that many more laboratories could be allowed to work on diagnosis. VECTOR reported having only a few persons trained to work on the live virus. In general there is a need for laboratory surge capacity that would need to be addressed.

The WHO stockpile of smallpox vaccine would, as in the case of other vaccines, be available within a week. It was noted that SAGE (Strategic Advisory Group of Experts on Immunization) judged that vaccination would be targeted to specific groups and not the entire population. Ensuring equitable and timely access to suitable medical interventions would be challenging in the case of very high demand. One expert mentioned that sanctions in Iran prevented the country from purchasing reference kits or other equipment for diagnostics, thus reducing preparedness capacity.

In a more general discussion, members of the IAG commented that the maintenance of the virus stocks after 1980 had led to today's situation in which the virus DNA has been sequenced and the sequence has been published. Consequently, as the SWG concluded, even if the virus stocks are destroyed today, the virus could potentially be recreated.

3. Implications

3.1 Risk of smallpox

Much of the discussion focused on the issue of risk. The nature of the risk of smallpox re-emergence has changed significantly over the past several decades. The IAG concluded that:

- The risk of variola virus re-emerging from natural sources is not likely to be greater today than in 1980, though it cannot be excluded.
- Following the eradication of smallpox in 1980, the risk of the disease re-emerging is likely to be from accidental or deliberate release of the virus from one or both of the two repositories or from other unknown locations.
- Additionally, the variola virus can now be recreated with synthetic biology and even modified. Many facilities worldwide – including some poorly regulated or unregulated laboratories – are thought to have the knowledge and expertise to do this. The nature of risk is evolving and is linked to the reduced cost of technology and ease of access to use it.

3.2 Implications for preparedness

Given the evolution in the risk from variola virus, the IAG concluded that preparedness and response must also change and that strategies are needed for risk reduction.

In theory smallpox is easy to control. However, experience has taught us that the control of epidemic diseases and the implementation of simple measures such as infection prevention and control can be challenging. In addition, the element of fear leads people to behave in irrational ways.

Emphasizing the importance of the core capacities required by the International Health Regulations (2005), the IAG agreed that smallpox risk reduction strategies should include:

- **Increased capacity for early detection**
  - increased clinical capacity to recognize and treat smallpox (e.g. develop and disseminate mobile telephone applications for photo recognition of skin diseases);
  - increased laboratory capacity, particularly at local level;
  - simple rapid diagnostic tests (such as dipstick diagnostics) for countries with low diagnostic capacity.
- **Increased capacity for disease control**
  - more public health countermeasures such as vaccines and antivirals;
  - reconsideration of the quantity of vaccines, diagnostics and antivirals likely to be needed in different scenarios; and
  - increased global preparedness against specific epidemic diseases such as smallpox.
- **Increased biosecurity**
  - revised regulations for research on DNA fragments of variola virus;
  - increased biosafety and biosecurity in all laboratories, including regular inventories of stocks;
  - strengthening of existing regulatory frameworks to ensure that they are applied correctly; and
  - coordination between sectors such as health, judiciary, law enforcement and the customs service.
- **More effective risk communication**
  - risk communication that acknowledges risk honestly, but puts it into perspective in comparison with other infectious risks;
  - transparency regarding dangers and what is being done to avoid or protect against them;
  - avoidance of attempts to mislead the public;
  - tracking of rumours (especially on social media) and responding to them quickly with factual information; and
  - community engagement in support of activities to detect and manage disease and to assist in sharing information.

3.3 Implications for research

A summary of the work of the WHO Advisory Committee on Variola Virus Research (ACVVR) was presented by its chair, Professor Geoffrey Smith of the University of Cambridge. After the declaration of smallpox eradication in 1980, the WHO Ad Hoc Committee on Orthopox Virus Infections recommended that live variola virus stocks should be destroyed. This resolution was passed by the World Health Assembly in 1996 and destruction was set for 1999. However, destruction was postponed so that essential research for public health benefit could be undertaken, under the oversight of the WHO ACVVR. There are only two WHO-sanctioned repositories of live variola virus in the world, CDC and VECTOR. Besides undertaking research with live variola virus, these institutions provide fragments of the virus to other laboratories that are governed by WHA resolutions. Reports of the ACVVR are provided regularly to WHO's governing bodies.

The research conducted under the auspices of the ACVVR has special importance. A number of IAG members urged that the research scope and terms of reference of the ACVVR should be reviewed to ensure that they take account of the new situation. They should also potentially be enlarged to include research on the efficacy of current vaccines and resistance to antivirals, biosafety and biosecurity, forensics in case of an outbreak, and scientific survey/monitoring. Professor Smith responded that any proposals for further research should be specific rather than general, and that the ACVVR scope is already sufficiently broad to take account of these issues.

WHO's recommendations on research with the live variola virus should be strengthened and given more emphasis. Some members even suggested that the recommendations against unauthorized use should be backed by sanctions. The IAG felt it was unlikely that any WHO Member State has adopted laws on the basis of the WHO recommendations so there is no effective sanction if they are contravened. Many persons in laboratories around the world may not even know of these recommendations.

There should be consideration of whether the risk of a smallpox outbreak is reduced by limiting variola research to only the two specialized WHO collaborating centre laboratories or whether risk would be further reduced by permitting live variola virus research in more laboratories. This would require increasing expertise and capacities, in addition to instituting appropriate regulations and regulatory mechanisms, especially by national authorities.

Some members felt that, despite the presence of resource persons, there was a lack of clarity regarding the types of research activities that require the live virus (i.e. in the WHO collaborating centres) and what activities can be done elsewhere or with non-live virus or DNA fragments. All variola research so far has been on the live virus or DNA fragments, so if we are to move to recommending research without the live virus we need to understand what this would involve and what the risks are.

Key points

- Adapted rules and regulations for manipulating and synthesizing variola virus genomes are necessary.
- There should be consideration of future research models, including the number of research sites and expertise development at global level.
- National public health laws should back WHO's recommendations concerning the distribution, handling and synthesis of variola virus DNA.

4. Way forward

Recognizing that the risk of smallpox re-emergence has increased with the low cost and widespread availability of technology to synthesize genomes, the IAG concluded that the WHO recommendations concerning synthesis and use of variola virus DNA fragments should be revised urgently.

The impact of synthetic biology goes beyond smallpox and could (some members felt “should”) be considered in relation to the eradication or elimination of other infectious diseases. Several members argued that, if there was a refusal to destroy the variola virus, there would be no destruction of any dangerous pathogen in the future. There was a call for a common policy for all diseases slated for eradication.

A comment was made that the history of epidemics shows that they are usually shaped by human activity – such as trade, travel, colonization, population movements etc. Synthetic biology is a new human activity and may result in its own spate of epidemics.

Annexes

Annex 1. List of participants

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Annex 2. Agenda

Consultation on the Public Health Implications of Synthetic Biology Technology Related to Smallpox

29–30 June 2015, World Health Organization, Geneva

Day 1, 29 June 2015		
9:00 – 9:30	- Introduction of members - Context and purpose of the consultation - Nomination of the Chair - Approval of the agenda	Dr K. Fukuda, WHO
9:30 – 9:35	Declaration of Interests	Ms R. Shademani, WHO
9:35 – 10:45	**Session 1: Current situation** - Overview of key concerns and issues including current approaches to preparedness and control (10 min) - Summary of the Scientific Working Group (SWG) report (10 min) - Discussion	Dr S. Briand, WHO Prof G. Smith, Chair of SWG
10:45 – 11:15	*Coffee break*	
11:15 – 12:30	**Session 2** - Scenario-based discussion of implications of synthetic biology	
12:30 – 13:30	*Lunch*	
13:30 – 15:00	**Session 2 - continued** - Continued discussion	
15:00 – 15:30	*Coffee break*	
15:30 - 17:00	- Continued discussion related to research	
Day 2, 30 June 2015		
9:00 – 10:30	**Session 3: Drafting of report**	IAG
10:30 – 10:45	Coffee break	
10:45 – 12:25	Continued drafting of report	IAG
12:25 – 12:30	Wrap up	Dr K. Fukuda, WHO

Annex 3. Report of the Scientific Working Group Meeting on Synthetic Biology and Variola Virus and Smallpox

Scientific Working Group Meeting

on Synthetic Biology and Variola Virus and Smallpox

16–17 April 2015

Geneva, Switzerland

Report to inform the Consultation

on the Implications of Synthetic Biology Technology on Variola Virus and Smallpox Control and Preparedness

Executive summary

A two-day Scientific Workgroup (SWG) meeting was called by the World Health Organization (WHO) to provide the scientific background information on synthetic biology technology on the variola virus, the causative agent of smallpox. The report from this meeting is to inform the meeting of an Independent Advisory Group on the Implications of Synthetic Biology Technology on Variola Virus and Smallpox Control and Preparedness. The meeting of the IAG will take place at WHO Headquarters, 29–30 June 2015.

As part of the background discussion, it was noted that smallpox was declared eradicated at the World Health Assembly in 1980 as a result of a global vaccination campaign in the 1960s and 1970s. Prior to eradication, smallpox was a highly contagious cause of illness and death and is estimated to have caused 300–500 million deaths during the 20th century.

At present, there are only two known and sanctioned collections of variola viruses present in two WHO collaborating centers (Centers for Disease Control and Prevention, USA, and State Research Centre of Virology and Biotechnology (Vector), Russian Federation). These repositories are for the purpose of research on diagnosis, treatment, and vaccine development.

The topics discussed during the meeting included:

1) an update on the pace of synthetic biology technology;
2) description of how variola virus could be synthesized and the availability of facilities, materials, and know-how required;
3) the implications of these technologies, including the potential for misuse;
4) modifications needed for current WHO recommendations on variola virus DNA; and
5) implications for the two WHO collaborating centres storing the stocks of variola virus.

The SWG concluded that with the increasing availability of DNA fragments that can be synthesized from simple chemicals, it would be possible to recreate the variola virus, and that it could be done by a skilled laboratory technician or undergraduate students working with viruses in a relatively simple laboratory. Because of the complexity of variola virus, it was felt that synthesis would require a deliberate act and sustained effort so was unlikely to be developed by accident. The SWG noted that although a recreated variola virus could be useful for research on vaccines and diagnostic tests, there are serious concerns regarding modifications of the virus that could be accomplished by institutions or individuals with malicious intent. The SWG concluded that the "WHO Recommendations Concerning the Distribution, Handling and Synthesis of Variola Virus DNA" should be revised. In addition, consideration should be given to adding a component or separate document on guidance to commercial DNA providers for screening requests for DNA fragments. Finally, as a result of being able to recreate variola virus using synthetic biology techniques, the destruction of the remaining stocks of variola virus at the two WHO collaborating centres would no longer be an irrevocable act. After destruction of the stocks, the variola virus could be recreated.

Introduction

At the World Health Assembly in May 2014, WHO was requested to undertake a consultation on the implications of synthetic biology on smallpox preparedness and control. A consultation process has begun with convening of a Scientific Working Group (SWG) on 16–17 April to deliver a scientific report on the state of the art of synthetic biology as applied to variola virus. It is composed of experts in poxviruses, biotechnology, and genetic engineering who were selected on the basis of their scientific expertise. Representatives from each of the two WHO repositories and a participant from a company commercially producing viruses participated as resource persons. The list of participants and declaration of interest are included at Annex I and Annex II, respectively.

WHO will then convene a meeting of an Independent Advisory Group (IAG) on the Implications of Synthetic Biology Technology on Variola Virus and Smallpox Control and Preparedness. This report summarizes the discussions of the SWG and is prepared to inform the IAG, who will meet on 29–30 June. The IAG will then draft recommendations to the WHO Director-General.

The specific objectives of the SWG meeting were as follows:

- Provide an overview of how variola virus might be re-synthesized and what facilities and materials would be needed to accomplish this(Part 1);
- Assess the availability of the technologies and capability to synthesize the variola virus (Part 2);
- Identify the implications of the technologies, both positive and negative, including the application and impact of synthetic biology on:
    - the development of new potential vaccines, diagnostic techniques and antiviral agents, and
    - the intentional misuse of variola virus (Part 3);
- Discuss the current "WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA" (Part 4);
- Consider the impact of the ability to re-synthesize variola virus on the retention of live variola virus stocks at the two WHO collaborating centres (Part 5).

Background

This background section has been added by the WHO Secretariat in order to provide a quick overview on the disease and its causative virus for non-specialized audience.

The last natural case of smallpox occurred in Somalia in 1977. The disease was declared eradicated at the World Health Assembly of 1980. Eradication was the result of an extended immunization programme tracking down the last case of the disease.

When smallpox was still affecting humans, 90% of cases were due to variola major ("ordinary" smallpox) with a historical high case fatality ratio sometimes reaching 30%. Smallpox was a systemic viral disease, generally presenting with a characteristic skin eruption. Smallpox was transmitted via respiratory droplets through prolonged face-to-face contact within 1.8 metres or by direct contact with infected body fluids (saliva) or fomites (contaminated objects, e.g. bedding, clothing, surfaces, etc.).

The strategy to control a smallpox outbreak included early isolation of cases (easily recognizable), contact tracing and early vaccination of contacts. The spread of smallpox was relatively slow and secondary cases mostly occurred within households and health care settings.

Since 26 October 1977, no cases were naturally observed. After the eradication of the disease, there have been only two known and sanctioned stocks of variola virus remaining in the world for the purpose of research on diagnosis, treatment and vaccine development. The known repositories of variola virus are located at two WHO collaborating centres (the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA, and the State Research Centre of Virology and Biotechnology (Vector), Koltsovo, Novosibirsk Region, Russian Federation). Although highly unlikely, re-emergence of smallpox could be due to:

- Persistence in the environment;
- Mutation of another orthopoxvirus;
- Laboratory release from the WHO repository laboratories;
- Re-creation of the virus with synthetic biology technologies.

Overview on synthetic biology technologies and variola virus

Variola virus is a large, complex double-stranded DNA virus and member of the larger group of poxviruses, called Orthopoxvirus. An important feature of the variola virus and all poxviruses is that the genome of the virus is non-infectious in isolation. To render this genetic material infectious, it must be introduced into a living cell that is infected with another poxvirus.

Laboratory studies on reactivating poxviruses date back to the 1930s.[1] Studies focused on the use of helper poxviruses to reactivate inactivated viruses – so-called non-genetic reactivation.[2] Later studies focused on "stitching together" fragments of virus genomes to produce large recombinant viruses.[3] Other developments involved inserting entire poxvirus DNA genomes into bacteria to allow for easier genetic engineering.[4] All of these developments advanced the study of the vaccinia virus and cowpox virus, poxviruses with similarities to variola. Progress in the chemical synthesis of DNA has led scientists to replace natural DNA segments or even whole genomes by chemically synthesized ones.

Recognizing the potential for reconstructing the variola virus using non-genetic reactivation and newer technologies, WHO has long placed restrictions on the amount of variola virus DNA that may be possessed by any laboratory other than the two collaborating centres. These limits have been set at less than 20% of the genome. Careful security controls have also limited access to the large DNA fragments and virus DNA stored at the collaborating centres.

Synthetic biology is the application of science, technology, and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms.[5] The dramatic advances and increasing commercial availability of synthetic DNA have resulted in an entirely new paradigm for "manufacturing" genetic material including making viruses "de novo". Key to this explosive development is use of an engineering–based approach, building on techniques of modern biotechnology and bioinformatics. [6]

There are several aspects of variola/smallpox regarding the impact of synthetic biology that warrant special attention. These include the fact that smallpox was officially eradicated, but variola virus is retained, and that vaccination against smallpox was discontinued in most countries by the 1970s. Thus a large proportion of the world's population has no immunity to the virus, and there is limited availability of medical countermeasures (i.e. diagnostic test kits, vaccines, and antiviral medications). Furthermore, the World Health Assembly has passed resolutions to destroy the two stocks of virus centralized in the WHO collaborating centres. Finally, advances in genetic engineering have enabled other poxviruses to be recreated, suggesting that variola virus too can be recreated.

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[1] Berry GP, Dedrick HM. A method for changing the virus of rabbit fibroma (Shope) into that of infectious myxomatosis (Sanarelli). J Bacteriol. 1936;31:50–1.
[2] Fenner F et al. Reactivation of heat-inactivated poxviruses: a general phenomenon which includes the fibroma-myxoma virus transformation of Berry and Dedrick. Nature. 1959;183:1340–1.
[3] Yao X-D, Evans DH. High frequency genetic recombination and reactivation of Orthopox viruses from DNA fragments transfected into Leporipoxvirus-infected cells. J Virol. 2003;77:281–90.
[4] Domi A, Moss B. Cloning the vaccinia virus genome as a bacterial artificial chromosome in Escherichia coli and recovery of infectious virus in mammalian cells. PNAS. 2002;99:12415–20.
[5] SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), SCCS (Scientific Committee on Consumer Safety), SCHER (Scientific Committee on Health and Environmental Risks), Synthetic Biology I Definition, Opinion, 25 September 2014.
[6] Carlson R. The changing economics of DNA synthesis. Nature Biotechnology. 2009;27:1092–4.

Part 1. How can variola virus be recreated using synthetic biology?

This process essentially involves three steps: synthesis, assembly, and reactivation (Figure 1). The genomes of more than 50 variola virus strains have been sequenced and are in the public domain. Although the sequence of the short, terminal, hairpin loops has not been determined for most sequenced variola virus genomes, at least one sequence has been determined and is in the public domain. Using published sequence information, DNA fragments of the virus can be synthesized in small pieces, then assembled step wise into larger pieces using in vitro assembly methods and finally assembled into full length genomes. Machines are now readily available to accomplish some of the steps of the assembly stage. DNA fragments used as the starting material can be ordered on the internet or synthesized locally. Of note, this gene synthesis technology can bypass any regulations about not making variola virus. For reactivation, large DNA fragments or entirely assembled genomes are introduced into a cell. A helper poxvirus must also be introduced in some cases to assist with the final assembly of the DNA pieces and in all cases to enable the reactivation of the full length DNA into live virus. For the reactivation step, helper poxviruses are easily available (e.g. fowlpox virus or myxoma virus).

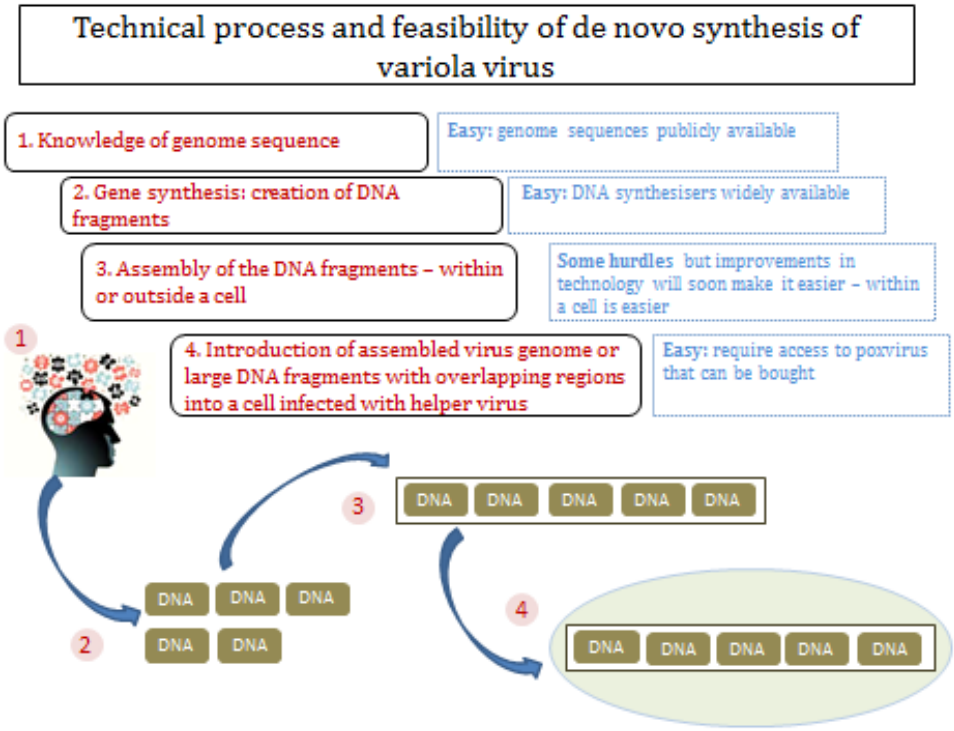

Figure 1. Technical process and feasibility of de novo synthesis of variola virus

Part 2. How easy would it be to recreate the variola virus? What physical facilities, resources, and know-how are needed?

Although it is possible to recreate variola virus, it is considerably more difficult than recreating other viruses, such as polio virus, influenza virus, or adenovirus. The recreation of variola virus would require a deliberate act and sustained effort. It is highly unlikely that this could occur by accident, and the WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA (Annex III) are designed specifically to prevent any such event. The recreation of variola virus is strictly prohibited.

In comparison to other viruses, the greater difficulty in recreating variola virus is due to:

- The larger genome size (186 kb versus 7.5 kb for polio virus or 36 kb for adenovirus 5)

- The ends of the double stranded DNA genome are linked by so-called terminal hairpins or telomeres into one continuous polynucleotide chain. This structure makes the re-creation of the virus significantly more complicated
- The fact that poxvirus DNA in isolation is non-infectious
- The need for a helper virus to infect the same cell in which the poxvirus genome is present.

Fragments of variola virus genome are available. Machines for DNA assembly are available and becoming cheaper.

More of resources and know-how needed include:

- Viable cells in which variola virus can grow
- Appropriate sterile growth medium, serum, antibiotic and other additives
- Knowledge of how to grow tissue culture cells using aseptic techniques to maintain these cells in a sterile environment
- Knowledge of transfection, the ability to introduce large DNA fragments into living cells
- Knowledge of how to grow and titrate the helper virus
- Knowledge of how to grow stocks of variola virus that are separate from the helper virus.

The minimum type of "facility" needed would include an incubator for the growth of tissue culture cells in which variola virus can replicate as well as, ideally, a microbiological safety cabinet in which cells could be handled (Level 2). All of this information is in the public domain. A skilled laboratory technician or undergraduate student with experience of working with viruses would be able to perform the above. The time required would be as little as three months. The cost of DNA fragments is decreasing rapidly, so the total cost similarly can be expected to decline.

Part 3. How does synthetic biology present increased dangers and challenges regarding variola virus? Are there some potential benefits of synthetic biology with regard to variola virus?

A number of serious modifications to the natural virus could be accomplished. These might involve:

- Inserting amino acid changes into viral proteins leading to resistance to antiviral treatment
- Making nucleotide changes such that current diagnostic kits would not detect the presence of the virus DNA, or distinguish it from other orthpoxviruses
- Changing the surface antigens of variola virus so that they would not be recognized as efficiently by immune cells or antibodies induced by the current vaccine, rendering the vaccination less effective
- Including additional genes that would enhance the virulence of the virus.

Benefits from the use of synthetic biology to recreate variola virus might include:

- Acceleration of the development of new vaccines
- Promotion of the study of the function of individual variola virus genes
- Facilitation of the production of materials needed for diagnostics.

The scale-up of synthetic biology and its increasingly widespread use introduces a large constituency that will need to be informed about the risks of working with variola virus and viral DNA fragments. Screening programmes of providers of DNA fragments and of consumers of DNA fragments will need to be developed and strengthened.

Part 4. Shortfalls of the current "WHO recommendations concerning the distribution, handling and synthesis of variola virus DNA" in light of the developments in synthetic biology?

The World Health Assembly passed resolutions (WHA52.10; WHA 55.15) that authorize temporary retention of the existing stocks of variola virus for the purpose of further essential research. As mentioned previously, the stocks are held in two known and sanctioned locations: CDC in the United States of America and Vector in the Russian Federation. The programme of research is overseen by the WHO Advisory Committee on Variola Virus Research, composed of members from

all WHO regions and advised by experts in public health, fundamental applied research and regulatory agencies.

Given the research activities, it was deemed necessary to regulate the undertakings through a policy and hence the development of "WHO Recommendations concerning the distribution, handling and synthesis of variola virus DNA." (Annex III) These were written with the scientific community, particularly orthopoxvirus researchers as the target audience. The recommendations were designed to restrict and control access to variola DNA to diminish the risk of any person or group creating variola or a variola-like virus, either intentionally or inadvertently.

According to these recommendations, the two laboratories that house these repositories may distribute variola virus DNA fragments (not exceeding 20% of the total viral genome) to requesting scientists, provided the recipients of the material adhere to the WHO recommendations. The two laboratories that house repositories are required to provide WHO with annual written and verbal reports regarding the use of live variola virus and the status of the repositories. The fundamental purpose of these recommendations was to ensure safety measures were respected to avoid any problems.

Today, the dramatic developments in synthetic biology necessitate a review and revision of the current WHO recommendations to ensure they are fit to serve their fundamental purpose. The SWG reviewed the current WHO recommendations. They argue that the purpose of these recommendations are to prevent the reconstruction of variola virus either through the reactivation of virus DNA or the accidental incorporation of variola DNA sequences into other orthopoxviruses. This purpose, the SWG proposes, should be made explicit in the recommendations document. The SWG suggests that there are several ways to prevent the reconstruction of variola virus. One is to limit the amount of variola virus DNA held by any one laboratory to an amount far less than a complete genome. The other way is to institute operations and practices that preclude any possibility of variola DNA coming in contact with another replication competent orthopoxvirus. As the capacity to synthesize the genes has improved to the point where genes and whole genomes can be synthesized, it is important to remember that clones encoding variola virus proteins should also be handled with the same restrictions in mind.

In the current climate of synthetic biology, it is recognized that the constituency for guidance is much broader. It includes national Ministries of Health, safety offices and legislative bodies, professional societies of scientists, specifically laboratory technicians and public health authorities, but also the institutions formal and informal that represent those involved in synthetic biology, including an unknown and presumably broad array of persons and institutions, commercial enterprises and individuals. A draft revision of these Recommendations is in Annex I at the end of this document.

In addition, it is worth considering adding a section to these recommendations or creating a separate document on screening guidance for providers of synthetic DNA. This would enable providers of DNA to screen orders and to address biosecurity concerns associated with the potential misuse of their products. It is recognized that such guidance cannot ensure that variola virus will not be synthesized by those unaware of, or choosing to ignore, such guidance.

Part 5. How does the ability to more easily recreate the variola virus impact on the retention of live variola virus stocks at the two WHO collaborating centres?

The immediate impact of being able to recreate variola virus using synthetic biology techniques is that the destruction of the remaining stocks of variola virus at the two WHO collaborating centres would no longer be an irrevocable act. After destruction of the stocks, the variola virus could be recreated.

Conclusion

With the rapid advances in synthetic biology, there is now the capability to recreate the variola virus, the causative agent of smallpox. While recreating variola is quite complex, the availability of genetic material, machines to do complex assembly, and increasing know-how among a broad array of persons, recreation may increasingly be possible. Furthermore, the rapid rise in availability of genetic material from commercial sources and the so-called grey market is driving the cost of this material down, making recreation possible by multiple institutions and persons, including those with malicious intent. The "WHO Recommendations concerning the distribution, handling and synthesis of variola virus DNA," should be revised. Consideration should be given to adding a component or separate document on guidance to commercial DNA providers for screening requests for DNA fragments.

With the development of these technologies, public health agencies have to be aware that henceforth there will always be the potential to recreate variola virus and therefore the risk of smallpox happening again can never be eradicated.

Annex I – List of participants

Members of Scientific Working Group

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Rapporteur

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Annex II. Declaration of Interests

The WHO consultation on the implication of synthetic biology on smallpox preparedness and control will be made through technical consultation with experts, including participants of the Scientific Working Group.

In accordance with WHO policy, all participants of the Scientific Working Group completed the WHO form for Declaration of Interests for WHO experts before start of the meeting. At the start of the consultation, the interests declared by the participants were disclosed to all consultation participants.

The participants declared the following personal current or recent (past 4 years) financial or other interests relevant to the subject of work:

David Evans is currently discussing a possible research contract with a private US company interested in synthesizing a poxvirus. The terms of the contract remain to be finalized. He also sits on the Federal advisory committee for the Canadian pathogens and toxins Act that will at some point need to discuss the implications of gene synthesis technology.

In response to employment and consulting, Robert Drillien has declared "employment and consulting on monkeypox and variola to Bavarian Nordic for an amount of 5000 euros for work conducted in 2011 and ceased in 2012."

Annex III. WHO Recommendations concerning the distribution, handling and synthesis of variola virus DNA

Based upon recommendations made to WHO by the WHO *Ad Hoc* Committee on Orthopoxvirus Infections (1990 & 1994) and the WHO Advisory Committee on Variola Virus Research (2003, 2004 & 2007)[7]
May 2008

Preamble

The only known stocks of variola virus are held at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States of America, and the Russian State Centre on Virology and Biotechnology (Vector), Koltsovo, Novosibirsk Region, Russian Federation, both of which are WHO (World Health Organization) collaborating centres. Any research using live variola virus has to be performed in the maximum containment laboratories of these institutions and requires permission from WHO. Genetic engineering of variola virus and attempts to produce live virus from DNA are strictly prohibited.

Scientists wishing to perform research on diagnostics or treatment of smallpox, or vaccines against smallpox, may obtain parts of the variola virus genome, which in its naked form is not infectious, from one of the WHO collaborating centres. WHO or the collaborating centres will advise scientists on the procedure to follow in order to obtain permission to receive viral DNA. Scientists should be aware that the amount of DNA they request or hold must not exceed 20% of the total viral genome (see also below).

The scientific community may not be fully aware that the distribution, synthesis and handling of variola virus DNA is governed by a series of recommendations made by the WHO Ad Hoc Committee on Orthopoxvirus Infections and by the WHO Advisory Committee on Variola Virus Research, which have been endorsed by WHO. Scientists wishing to obtain, handle or synthesize variola virus DNA must therefore comply with these recommendations. The present document gives an overview of these recommendations, which are reproduced in their original wording as found in the various WHO meeting reports (http://www.who.int/csr/disease/smallpox/research/en/index.html).

Distribution of variola virus DNA

The two WHO collaborating centres acting as repositories for variola virus may distribute variola virus DNA fragments to appropriate research laboratories that request them provided that:

a) The request has been submitted to the international repository through WHO/Headquarters (*1, 2*).
b) The receiving laboratory agrees that the DNA will not be distributed to third parties, unless authorization by WHO has been obtained. This should be controlled through Material Transfer Agreements between the distributing and receiving laboratories (with copy to WHO) (*1, 2, 3*).
c) An annual report on the status of the variola virus DNA will be made to the international repository and to WHO (*2*). No laboratory (except the international repositories) shall be permitted to hold clones representing more than 20% of the variola virus genome at any one time (*2*). Fragments of variola virus DNA, not exceeding 500 base pairs in length, may be freely distributed between identified laboratories for use as positive controls or standards in diagnostic kits, providing collectively they do not exceed 20% of the total genome size (*4, 5*).

[7] Accessible at http://www.who.int/csr/disease/smallpox/SummaryrecommendationsMay08.pdf

Handling of variola virus DNA

Studies on variola virus DNA are permitted on condition that:

a) The DNA will not be used for insertion into vaccinia virus or related poxviruses (*2*).
b) All work with variola virus DNA (greater than 100 nucleotides long) is done following a written risk assessment and in accordance with locally agreed national guidelines (*2*).
c) No other orthopoxviruses are handled in the laboratory rooms where variola virus DNA is studied (*2*).
d) All by-products containing variola virus DNA are disposed of by autoclaving at 120°C for 30 minutes (*6*).

Synthesis of variola virus DNA

a) Attempts to synthesize full-length variola virus genomes or infectious variola viruses from smaller DNA fragments are strictly forbidden (*7*).
b) In vitro synthesis of variola virus DNA, or any DNA encoding a variola virus polypeptide, where the length of the DNA exceeds 500 base pairs requires approval from WHO. Similarly, mutagenesis of orthopoxvirus DNA of larger than 500 base pairs, with the aim of producing the corresponding variola virus DNA sequence, again requires permission from WHO. Under no circumstances can laboratories, other than the WHO collaborating centres hosting the variola virus repositories, hold DNA comprising more than 20% of the total genome (*4, 7*).
c) Production of DNA microarrays, on which small oligonucleotides (less than 80 base pairs) are covalently bound to a matrix and which, in aggregate, may span the entire genome, does not require permission from WHO (*4, 5*).

Reporting obligations

Variola virus DNA is distributed to scientists on the understanding that an annual report on the status of variola virus-specific DNA clones will be made to the international repository (see above: Distribution of variola virus DNA, paragraph c). This reporting obligation also applies to scientists who have obtained permission from WHO to synthesize variola virus DNA larger than 500 base pairs, or generate variola virus-like DNA by site-directed mutagenesis of other orthopoxvirus DNA.

References

1 Report of the *Ad Hoc* Committee on Orthopoxvirus Infections, 1990, page 5.
2 Report of the *Ad Hoc* Committee on Orthopoxvirus Infections, 1994, page 8.
3 Report of the WHO Advisory Committee on Variola virus Research, 2007, 23.4
4 Report of the WHO Advisory Committee on Variola virus Research, 2003, 11.7
5 Report of the WHO Advisory Committee on Variola virus Research, 2004, 8.2.
6 Report of the Ad Hoc Committee on Orthopoxvirus Infections, 1994, page 9.
7 Report of the WHO Advisory Committee on Variola virus Research, 2004, 8.4.