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Adventitious Agents and Live Viral Vectored Vaccines: Considerations for Archiving Samples of Biological Materials for Retrospective Analysis

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1. The need for archiving vaccine samples and other biological materials

Vaccines are one of the most effective public health medicinal products with an excellent safety record. Well-planned and implemented immunization programs have profoundly reduced the morbidity and mortality of targeted diseases [1], such as the global eradication of smallpox [2] and the elimination of poliomyelitis [3] and measles [4] from many regions of the world. Since vaccines are usually administered to large populations of healthy people including children, frequently with the goal of near universal coverage (under legal mandate in some countries), their safety and quality are paramount for public health.

As vaccines are produced using biological materials, there is a need to safeguard against potential contamination with adventitious agents. Adventitious agents are defined by the World Health Organization (WHO) as microorganisms that may have been unintentionally introduced into the manufacturing process of a biological medicinal product [5]: these

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include bacteria, fungi, mycoplasma/spiroplasma, mycobacteria, rickettsia, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents and viruses. Adventitious agents could be inadvertently introduced into a vaccine through starting materials used for production, such as cell substrates, porcine trypsin, bovine serum, or any other source materials of animal or human origin [6]. Therefore, extensive testing is recommended at various stages during vaccine manufacture to demonstrate the absence of adventitious agents [5]. Additionally, the incorporation of viral clearance steps in the manufacturing process, which evaluate the capability of the manufacturing production process to inactivate and/or remove potential viral contaminants [7] can aid in reducing the risk of adventitious agent contamination in a biological product; however, for live viral vaccines, aside from possible purification of the virus or vector, extensive adventitious agent clearance may not be feasible. Hence, the issue of unknown contamination risks of live or vectored vaccines requires more stringent safety oversight [5].

In the event that an adventitious agent is detected in a current vaccine, it is important to determine its origin, evaluate its potential for human infection, and discern which batches of vaccine may have been affected for notification and in order to take risk management action plans. To achieve this, it is necessary to have archived samples of the vaccine and ancillary components, ideally from developmental through to current batches, as well as samples of the biological materials used in the manufacture of the vaccine, since these are the most likely sources of an adventitious agent.

Although currently recommended testing has a good record for demonstrating absence of adventitious agents in vaccines, there have been rare cases of adventitious agent detection in some licensed vaccines. A recent notable event was that of porcine circovirus 1 (PCV1) in a rotavirus vaccine [8–10]. Early episodes of contamination of biologicals (e.g., tetanus contamination of diphtheria anti-toxin) date back to the beginning of modern immunization and led to the establishment of regulatory oversight in the early 1900’s [11]. The discovery that early polio vaccine was contaminated by simian virus 40 (SV40) due to infection of rhesus monkeys resulted in a major manufacturing change in the cell substrate from primary rhesus monkey kidney cells to African Green monkey kidney cultures [12]. The detection of bacteriophage was detected in measles and polio vaccines, reverse transcriptase in measles and mumps vaccines, and the emergence of bovine spongiform encephalopathy (BSE) commonly known as “mad cow disease” in the 1980’s, and ultimately the human version variant Creutzfeldt-Jakob disease (vCJD) in the 1990’s, led to considerable regulatory deliberations, and also guidance on the use of bovine (and other) materials that could transmit transmissible spongiform encephalopathies (TSE’s) [12–15].

Viral vaccines are grown in cell cultures that may have been propagated in media containing bovine serum, and possibly used porcine trypsin for cell passage. Thus, in addition to archiving final released vaccine, there is also an argument for archiving the starting biological materials and of records that provide full traceability of biological materials used in vaccine manufacture. However, the issue of cell and serum archiving and their full traceability are not within the scope of this document at this point in time, and this paper will focus on the live recombinant viral vectored vaccine itself.

Laboratory testing is used to demonstrate the absence of adventitious agents in the vaccine. In the event that contamination is found in a released vaccine after it has been marketed, samples obtained from vaccinees (e.g serum and PBMCs) may be used to evaluate whether the adventitious agent infected the vaccine recipient. Retrospective testing confirmed the presence of PCV1 DNA in Rotarix® since the initial stages of its development and in vaccine lots used in clinical studies conducted pre- and post-licensure [10]. Therefore, adventitious agents that fail detection using technologies available at the time a vaccine was originally produced and used, might at a later stage be detected by re-testing using emerging technology. In order for a new technology to be utilized to improve vaccine safety and detect past contamination events, samples of the vaccines and materials used in their production and samples from the vaccine recipients need to be collected and archived. Hitherto, the need for formal guidance on such vaccine sample archiving has been recognized but not fulfilled [15]. The Brighton Collaboration Viral Vector Vaccine Safety Working Group, formed in 2008 with voluntary representatives from academia, government and industry [16], has therefore summarized in this paper several prior major cases of vaccine contamination and provides points for consideration on sample archiving of live recombinant viral vector vaccines in humans. The Group recognizes that this document may be controversial, especially the cost implications, but feel it is important to stimulate the discussion on both the need for vaccine sample archiving and how this need might be met.

While this document focuses on live viral vector vaccines, relevant past experience with traditional viral vaccines are discussed and the lessons learnt may be usefully applied to novel vaccines, especially those that are live attenuated.

2. Historical context: past to future

History has shown that extensive testing for adventitious agents during manufacture of vaccines has prevented major contamination events and potential adverse clinical consequences. However, reports of product contamination have occurred periodically, mostly due to viruses present in biological reagents used for production (e.g. animal tissues or primary cell substrates, serum, or trypsin). The genomic and biotechnology revolution of the last decades has enabled the development, licensure, and production of many new vaccines and biologicals. The number of vaccine manufacturers who supply the global market has also been increasing, many of whom are from emerging economies [17]. While all vaccine manufacturers are regulated by their national health authorities, and those who supply UNICEF are pre-qualified by the WHO as meeting good manufacturing practice (GMP) standards [18], their capabilities differ and many need improved pharmacovigilance systems, such as standardization of safety reporting [19].

Since many if not most vaccines globally will likely continue to be made using biological reagents for the foreseeable future, the possibility of adventitious contamination cannot be totally excluded. Therefore, it is important to consider prospective sample archiving of vaccines and the use of new technologies and knowledge to test for contamination as they become available.

To provide the context for and to better illustrate the need for this document, we have reviewed several notable contamination events and the resulting corrective regulatory actions. These events have also been reviewed in detail elsewhere. [12, 20, 21]

2.1 SV40 contamination of polio vaccine

2.1.1 Discovery of SV40 contamination in polio vaccine—SV40 is a monkey polyoma virus that was discovered in 1960 and can induce tumors in rodents and transform human cells in culture [22]. The Salk inactivated polio vaccine (IPV), first licensed in 1955 in the U.S.A., was made in primary rhesus monkey kidney cells. It was already in wide use in 1961 when it was discovered that some of the vaccine lots were contaminated with SV40. At least 10–30 million persons were estimated to have been exposed to SV40-contaminated polio vaccine in the U.S.A. [23]. Testing of stored U.S. samples from vaccine lots produced in 1955 showed that the levels of SV40 were inconsistent across vaccine lots with some uncontaminated lots [24]. However, as samples of vaccine lots produced were not archived during 1955–1961, the period of likely SV40 contamination, no further testing was possible.

Since the early 1960's, polio vaccines have been tested for SV40 infectivity in cell cultures. In a retrospective UK study, PCR was used to examine archived samples of oral polio vaccines (OPV) dating from 1966 to the time of the study (1999), including all vaccines used in the UK since 1980, for the presence of SV40 sequences [25, 26]. Of 132 materials examined, 118 were negative on initial testing and fourteen gave reactions which on further examination were attributed either to cross contamination during handling in the laboratory at the National Institute for Biological Standards and Control (NIBSC), UK or to non-specific amplification; it was concluded that none of the samples contained SV40 sequences [25]. Some polio vaccines prepared from 1954 to 1961 were contaminated with infectious SV40. It has been assumed that all polio vaccines were SV40 free in the United States after 1961 and in other countries after 1962. Following a WHO requirement [27] that was prompted by the detection of SV40 in some human tumors, [28] a multilaboratory study was conducted to test SV40 polio vaccines prepared after 1961. Vaccine samples from 13 countries and the WHO seed were tested. All vaccines were SV40 free, except for vaccine from a major eastern European manufacturer whose inactivation procedure failed to completely inactivate SV40 in OPV vaccine seed stocks [29].

In Sweden, US-produced polio vaccine was used in 1957; but from 1958 on, only Swedish produced vaccine was used. Testing for SV40 began in 1961, including retrospectively of vaccines produced earlier, but there is doubt as to the validity of the negative results [25, 26].

Multiple epidemiologic studies have been conducted to assess the long term effects of SV40 contaminated vaccine in humans [15]. More recently, there was concern that although SV40 infection alone is unlikely to cause mesotheliomas and brain tumors in which SV40 genetic sequences had purportedly been detected, it may have acted as a cofactor in the pathogenesis of some tumors, with co-carcinogenicity between SV40 and asbestos being of particular concern [30]. However, in an extensive review by the US Institute of Medicine (IOM) in 2002, it was concluded that these studies were "sufficiently flawed" so there was insufficient evidence to determine whether SV40-contaminated polio vaccine caused cancer or not [15].

2.1.2 Regulatory actions taken after SV40 contamination of poliovirus—In 1989, the WHO developed guidelines that required monkeys to be free of SV40, a practice already implemented in many countries. Validated nucleic acid amplification tests are generally now used to determine that virus seed lots used to produce viral vaccines are specifically free of SV40, along with a tissue culture test in Vero cells [5, 31]. Worldwide, manufacture of the vaccine was changed to African green monkey kidney cells, since this species is generally free of SV40. Authorities worldwide require all licensed vaccines to fulfill general safety, sterility, and purity requirements [32].

The 2002 IOM report recommended that federal agencies develop a 'Vaccine Contamination Prevention and Response Plan' which would include "strategies for routine assessment of vaccine for possible contamination; notification of public health officials, health care providers and the public if contamination occurs; identification of recipients of contaminated vaccines; and surveillance and research to assess health outcomes associated with the contamination" [15]. It also recommended considering a program to store samples from each vaccine lot approved for release in order to make it possible to test for contaminants if new detection methods become available or safety questions arise well after the vaccine has been used. Currently, manufacturers are required to store samples of each released lot only until one year following the expiration of that lot [32, 33].

2.2 Contamination of yellow fever vaccine

2.2.1 Avian retrovirus contamination of yellow fever vaccine—Avian leucosis virus (ALV) is an exogenous retrovirus that causes leukemia in chickens by means of insertional activation of cellular oncogenes [34]. The yellow fever (YF) vaccine comprises the 17D attenuated strain and is propagated in chicken embryos by inoculation of 7 to 9 day old embryonated eggs with the vaccine strain. The 17D YF vaccine became the main means of protection for travelers and those in the military during World War II [35] and was received by over one third of the US Army [36]. ALV contamination of the YF vaccine was first discovered in 1966 and concern arose about the possible oncogenic risk among former military vaccinees [36]. Waters et al. conducted a retrospective case control study, examining record-documented YF vaccination history during World War II among representative sample of 2,659 veterans who died of various specific cancers between 1950–1954 or 1959–1963 and age-matched controls [36]. The study found no suggestion of association between the vaccine and cancers as classified, despite good statistical power. However, this study could only examine cancers with a latent period between 5 and 22 years, and failed to detect any elevated risk of hepatic neoplasia among vaccinees with prior history of serum hepatitis (see 2.3.2).

More recently, YF vaccines produced by three manufacturers were all found to have endogenous avian retrovirus (EAV) particles and endogenous avian leucosis virus (ALV-E) particles, which originate from ancient retroviral sequences and from a nonpathogenic ALV, respectively, that exist as a normal part of the chicken genome (discussed in 2.4 below). The absence of evidence of infection with ALV-E or EAV in 43 YF vaccine recipients suggests a low risk, if any, for transmission of these viruses [37].

2.2.2 Hepatitis B virus (HBV) contamination of yellow fever vaccine—

An epidemic of icteric hepatitis in 1942 affected approximately 330,000 U.S. Army personnel. This outbreak was linked to specific lots of YF 17D vaccine stabilized with human serum that retrospectively was found likely to have been contaminated by HBV [35, 38, 39]. The outbreak was controlled by shifting to a new serum-free YF vaccine. However, the link between the hepatitis and the YF vaccine was not proven until a 1985 study in which 597 veterans who had been in the army in 1942 were interviewed and serologically screened. They were categorized in three groups: the first group included patients who had jaundice after having received the vaccine, and 97% of them were positive for antibodies to HBV. The second group contained those who had received the vaccine but did not fall ill, 76% of whom had positive HBV antibodies. The third group consisted of persons who received a serum-free vaccine and did not have jaundice; 13% of them had positive antibodies to HBV, similar to the prevalence in the general US population [35, 38, 39]. Together these results suggested that the YF vaccine transmitted HBV.

2.2.3 Regulation resulting from ALV and HBV contamination of YF vaccine—

Extensive testing is recommended to assure vaccine safety; only a few cases of unexpected viruses have occurred but they highlight the importance of adventitious agent testing for all biological materials that are used for vaccine production. Although there is no evidence for human disease associated with ALV, all countries now use seed virus prepared in specific-pathogen free (SPF) eggs that are free from ALV as indicated by WHO [40, 41]. However, some permit the production of vaccine in embryonated chicken eggs that may contain ALV, but need justification due to cost and difficulty in procuring ALV-free eggs that would result in restricting availability of the YF vaccine. For this reason, the revised WHO Requirements for YF vaccine do not require ALV-free eggs. It should be noted that the WHO requirements regarding YF vaccines are not mandatory and approval for use is controlled by individual nations [38, 40, 42]. Accordingly, the vaccines, particularly with respect to their quality control, can vary.

HBV contamination in the early lots of YF 17D vaccine due to pooled human serum that was used as a stabilizer resulted in the elimination of human serum from YF vaccines.

2.3 Endogenous avian retroviral particles in MMR vaccines

In 1996, reverse transcriptase (RTase) activity, an enzyme typically associated with retroviruses, was detected in chicken cell-derived measles and mumps vaccines [43]. The RT activity was found to originate from the chicken embryonic fibroblasts used as a substrate for vaccine manufacture and was associated with virus-like particles containing endogenous retrovirus sequences (EAV) that are normally present in the host genome. Infectivity studies demonstrated these particles were non-infectious in a variety of human cell lines [42, 44, 45]. Although EAV and also endogenous avian leukosis virus (ALV-E) RNA sequences were reported in MMR vaccines, there was no evidence of transmissibility of ALV and EAV sequences to MMR recipients [40]. Pre- and post MMR vaccination samples from 33 children as well as samples from randomly selected blood donors were tested for ALV and EAV sequences. Despite the use of a highly sensitive PCR assay none of the samples tested were positive for either ALV or EAV sequences [40]. Various studies did not reveal any

adverse effects of the presence of these sequences or of RTase activity in chicken cell derived vaccines and the WHO determined that the overall benefit/risk balance remains highly in favor of continued use of the vaccines [12, 46].

2.4 PCV-1 contamination of vaccine

Porcine circoviruses (PCVs) are small non-enveloped virus containing a single-strand circular DNA genome virus. Two antigenically and genomically distinct variants exist in the swine population worldwide: PCV1 is non-pathogenic for pigs; PCV2 has been associated with various porcine disease syndromes [47].

PCV contamination of a vaccine was first discovered by Victoria et al. [8], while experimenting with new methods for detecting adventitious viral contamination. Using metagenomics and a pan-microbial microarray (versus a more traditional method of viral species-specific PCR), a panel of eight live attenuated vaccines that included oral polio virus, rubella, measles, yellow fever, human herpesvirus 3 (HHV-3), rotavirus, and multivalent measles/mumps/rubella were analyzed. In one orally administered rotavirus vaccine the metagenomics study uncovered a complete porcine circovirus-1 (PCV1) genetic sequence. Follow-up studies indicated that the number of PCV1 viral particles present in the vaccine was about the same as the number of rotavirus vaccine particles [48]. The contaminant was subsequently easily detected by virus-specific PCR; this had never been previously applied, because this agent, not being of concern to the swine industry, was not specifically included in the testing recommended for porcine viruses [49] and in tests recommended for extraneous agents [50]. No other microbial genetic sequences were detected in the study, that had not been previously uncovered in any of the vaccines.

In cell cultures, although PCV gene expression and replication takes place in human cells, the infection is non-productive [9]. Furthermore, PCR screening of a variety of different human cell lines, including human tumor cells, demonstrated that PCV1 was not generally prevalent in commercially-available cell lines [8]. Epidemiological data for humans show ambivalent results for serum antibody to PCV1 and current PCV1 knowledge is sparse and contradictory [51, 52].

2.4.1 PCV-1 in Rotarix®—The rotavirus vaccine contaminated with PCV1 described above was Rotarix®, an oral rotavirus vaccine manufactured by GlaxoSmithKline (GSK), first licensed in Europe in 2006 and US in 2008. Two doses of the vaccine are given to infants beginning at six weeks of age to protect against gastroenteritis due to rotavirus infection. The WHO estimates that rotaviruses are responsible for approximately 500,000 deaths each year, with more than 85% occurring in low-income countries in Africa and Asia.

Upon being informed of the PCV1 contamination of Rotarix®, GSK rapidly initiated an investigation to confirm the source, nature and amount of PCV1 in the vaccine manufacturing process and to assess potential clinical implications of the finding. The investigation also considered their inactivated poliovirus (IPV)-containing vaccines, since poliovirus vaccine strains are propagated using the same cell line as the rotavirus vaccine strain. Results confirmed the presence of PCV1 DNA and low levels of PCV1 viral particles

at all stages of the Rotarix® manufacturing process. PCV1 DNA was not detected in the IPV-containing vaccine manufacturing process beyond the purification stage.

GSK subsequently notified regulatory health authorities about the discovery of PCV1 in Rotarix® and conducted additional studies confirming that PCV1 DNA was present in both the finished Rotarix® vaccine, in vaccine lots used in clinical studies, and in the source cell bank and master seed; the latter findings suggesting that the PCV1 contamination occurred during the early stages of vaccine development [10, 53]. The contamination was believed to have derived from the use of contaminated porcine trypsin in the development and manufacture of the vaccine.

Rotarix® is widely used globally in both developed and less developed settings. At the time of discovery of PCV1 contamination, ~100,000 children had received the vaccine during clinical trials and ~68 million doses had been distributed worldwide. Therefore, due to the potential public health impact, regulatory agencies further examined the state of the contamination.

2.4.2 Regulatory Actions Taken for PCV1 Contamination of Rotarix®

2.4.2.1 European Union: In the European Union, Rotarix® is available in all Member States, but is usually not part of their routine childhood vaccination schedules.

After GSK notified the European Medicines Agency of the unexpected presence of PCV1 DNA in batches of Rotarix® in March 2010, its Committee for Medicinal Products for Human Use (CHMP) initiated a review. In view of the ubiquitous presence of the virus in food, the oral route of administration of the vaccine (mimicking the route of natural exposure), and the absence of both known pathogenicity and serious adverse reactions reported with the vaccine, the Committee concluded in March 2010 that the findings do not present a public health threat and vaccine usage should continue [52].

A formal review of Rotarix® was also initiated by the European Commission, which concluded that the vaccine continues to have a positive benefit-risk balance and that the presence of a small amount of PCV1 viral particles does not present a risk to public health. However, since PCV-1 should not be present in the vaccine, it was incumbent upon the manufacturer to propose measures of manufacturing the vaccine free of the virus, although such measures would take time to implement [53].

2.4.2.2 United States- FDA: An initial review of data on the presence of DNA from PCV1 in Rotarix® was performed in March 2010. The FDA similarly concluded that there was no evidence that the presence of PCV1 DNA in Rotarix® posed a safety risk and confirmed the excellent safety record of the vaccine [10]. Nevertheless, the FDA recommended that clinicians temporarily suspend use of vaccine until the Agency learned more. On 14 May 2010, after discussions in the FDA Vaccines and Related Biological Products Advisory Committee, the suspension of the use of Rotarix® was removed. The decision was based on an evaluation of information from laboratory results from the manufacturer and the FDA's own laboratories, a thorough review of the scientific literature, and input from scientific and

public health experts, including members of the FDA's Vaccines and Related Biological Products Advisory Committee that convened on May 7, 2010 to discuss these vaccines.

The Agency's decision was further based on the strong safety records of the vaccine, the lack of evidence that PCV1 or PCV2 cause infection or disease in humans, and the substantial benefit of the vaccine in preventing death in some parts of the world and hospitalization for severe rotavirus disease in the United States. These benefits outweighed the theoretical risk posed by the presence of PCV1 in the vaccine [54].

Since the investigation into the PCV1 contamination of Rotarix® by GSK and federal agencies, PCV1 has continued to be researched and manufacturing procedures have been further developed. It was found that PCV1 could infect human hepatocellular carcinoma cells. Although the author emphasizes that the connection between this evidence and vaccine safety is unclear, it does demonstrate that a negative cell culture may not give the full scope of the contaminant's capabilities [54]. The presence of PCV1 early in the vaccine production process has also triggered further research on contaminants in cell culture, and material used in the manufacturing process such as bovine serum and trypsin [55]. Furthermore, research is being done to improve the manufacturing procedure by creating a new quantitative tool to detect residual porcine DNA [56].

No PCV1 DNA was detected in a separate and widely used rotavirus vaccine, Rotateq™, manufactured by Merck, although sensitive assays detected small fragments of PCV2 genomic DNA. It was determined that these were of no consequence to the safety of the vaccine and no regulatory action was taken.

3. Existing guidelines to assure viral safety

Strict measures are currently in place to assure the safety of vaccines as well as all other biological medicines. For example, the US Code of Federal Regulations defines product safety as "the relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time [32]". The two critical components of safety are sterility, which is defined in 21CFR600.3(q) as "freedom from viable contaminating microorganisms"[32], and purity which is defined in 21CFR600.3(r) as the "relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product to meet the requirements of 610.13" [55]. Vaccines are currently tested using a variety of assays to demonstrate safety and purity, including specific and general assays for detection of potential contaminants, and there may be a need to consider new technologies that become available for broad detection of unknown agents as well.

Nevertheless, while materials and culture processes leading to medicinal products are tested to demonstrate the absence of adventitious agents, there might be occasional unintended introduction as demonstrated by the PCV1 situation. Reports indicate that adventitious agent contaminations are more frequently caused by bacteria or mycoplasmas, which are more easily detected, than by a virus. The safeguards against viral contamination include

implementation of GMP, thorough testing or use of certified raw materials, viral safety evaluation at critical production stages (e.g. virus seeds and virus harvests) and validation of the viral clearance capacity (if any) of the downstream purification process [57].

National and international regulatory authorities provide guidelines on the manufacturing, standardization and quality control of medicines [32]. These are subject to continuous review and modification to reflect the current state of science and technology.

However, for live viral vaccines, in-process adventitious agent inactivation steps are not part of the manufacturing process, since these steps would most likely compromise vaccine viability and immunogenicity. While inactivated vaccines include a vaccine virus inactivation step as part of the manufacturing process, the ability of that step to inactivate potential adventitious agents is often not evaluated, particularly for products that have been on the market for some time. For newer or investigational vaccines, an inactivation step(s) that assesses the ability to inactivate a variety of agents should be part of their manufacture. The safety of live viral vaccines has to be assured by direct testing of the vaccine and of materials used in its manufacture, and to use control cell cultures for demonstrating that batches of cells or eggs cultivated in parallel to those used in vaccine manufacture but not infected with the vaccine virus, show no signs of infection by other agents. It is important to include a risk assessment process in the overall viral control strategy used during the manufacture and testing of vaccines. The risk assessment is necessary to identify potential sources for entry of adventitious agents into the vaccine, and to develop a strategy to mitigate the risk of adventitious agent introduction. The risk assessment can be used to tailor the biosafety testing that is performed on raw materials, vaccine seeds, vaccine bulk materials and final product [58]. This is an evolving field and regulatory agencies are developing regulations regarding using new detection technologies to evaluate future vaccines for adventitious agents.

4. Methods for Developing Proposed Considerations

A Brighton Collaboration Viral Vaccine Vector Safety Working Group was formed in 2008 with about 30 expert members. The group consists of persons with expertise in virology, regulation and vaccine safety, and meets via monthly conference calls. The development of this considerations paper was based upon literature review, a systematic review of current regulations from both Europe and United States and group consensus. Outside experts on sample archiving were invited to contribute as needed.

4.1 Lessons Learned from Past Contamination Events

Contamination events have invoked considerable discussions in industry and regulatory agencies leading potentially to implementation of risk mitigation strategies or formulation of new recommendations [59]. However, the issue of comprehensive storage or archiving of vaccine samples so that the origin of any future contamination event can more easily be traced and corrective action taken has not been addressed. As the past examples of contamination events demonstrate, it is important to archive samples consistently for an extended period of time so as to allow future researchers to determine the extent and impact of any contamination. Some potential adverse events induced by an adventitious agent (e.g.

cancer) can occur many years after vaccination and the period of archiving should reflect this scenario. The ALV contamination of the yellow fever vaccine was examined using available information on vaccinations, which came from a cohort that was not likely to be vulnerable to infection or to be immunocompromised, and so is not easily generalizable. With the HBV contamination of the yellow fever vaccine, there was a problem with obtaining historical samples and it was a challenge to use these samples due to the lack of guidance for sample archiving at the time they were prepared for storage. As occurred during the investigation of possible adverse events resulting from SV40 contamination of the polio vaccine, existing samples were not representative of the distribution of the vaccine and epidemiologic studies able to be performed with existing samples were flawed, preventing a concrete conclusion [15]. The existing samples were also precious, which led to problems of establishing acceptable protocols to extract DNA, and difficulties may have resulted in some initial cross contamination. The extended storage of vaccine samples would assist future researchers to identify contaminated vaccine lots, and so determine a more accurate relative risk for specific populations.

The value of a centrally organized sample archive was illustrated during a relatively recent investigation of 1976–77 swine influenza vaccine to assess if the still unexplained elevated risk of Guillain-Barre syndrome (GBS) encountered with this vaccine was due to vaccine contamination by Campylobacter, a now known cause of GBS and endemic in poultry, from which eggs used for influenza vaccine production are sourced [15, 60]. By the time this hypothesis was formulated in 2006, however, some thirty years after vaccine production, there was extreme pessimism that vials of the original vaccine from different manufacturers and lots kept frozen throughout, could be found. Fortuitously, after considerable effort and a nationwide search, influenza researchers at Baylor University were found to have such an archive, thereby allowing this hypothesis to be tested, and ultimately rejected. These experiences highlight the need for the development and implementation of standard procedures for sample archiving, including guidance in the collection, preparation and storage of samples.

4.2 Potential safety concerns related to novel viral vaccines

The development of some novel viral vaccines have necessitated the use of human tumorigenic and tumor-derived cell substrates, which could pose additional safety concerns related to the potential presence of unknown tumor viruses and latent viruses that may not be detected by the currently recommended assays [61]. Additionally, the use of large virus vectors can provide a target for endogenous retrovirus integration and amplification in the vector virus [62–65]. Therefore, advanced nucleic acid technologies with broad virus detection are being investigated for cell substrate characterization and may also be useful for characterization of the virus seed or products.

5. Avoidance of Adventitious Agents

The production of live virus vaccines involves propagation of the vaccine virus in a suitable cell culture system, possible cell disruption for maximal yield of virus and, if necessary and if possible, purification of the virus. For biological products such as live virus vaccines, the

introduction of an inactivation step(s) for adventitious agents as part of the downstream manufacturing process is not possible, since such a step is likely to compromise the immunogenicity of the vaccine virus. Thus the use of well characterized cell bank systems and qualified reagents for production is an even more important step to assure vaccine safety compared with their use for other vaccines or biological medicines. Progress has been made in the development of serum-free media for cell growth needed for the production of viral vaccines. However, the risk of introduction of adventitious agents through the use of other animal-derived substances such as trypsin during the production process remains. Use of gamma-irradiated or UV-treated reagents is also being considered in some cases when there is no adverse effect on the cell substrate. The risk of adventitious agents is reduced by current viral safety testing regulations and measures that recommend redundancy in testing using different assays and at different stages in manufacturing. Therefore, although the risk of adventitious agent introduction using primary cell substrates such as eggs and primary tissue cultures is higher than using a well characterized cell line, extensive and redundant testing provides confidence for their deployment during vaccine manufacture. Although complete elimination of animal derived reagents from the manufacturing procedure leads to a substantial reduction of the risk of contamination, the risk cannot be completely eliminated since animal-derived raw materials might be used in the production process of non-animal derived raw materials, such as enzymes to digest proteins to peptides and amino acids. Additionally, some cell substrates may not adapt to serum-free growth conditions. Further, it may be possible for viral contamination to arise from chemical reagents for growth medium preparation as illustrated by the minute virus of mice (MVM) contamination incident in the manufacture of a biopharmaceutical product [66].

On the other hand, advanced nucleic acid based technologies that have demonstrated success for detection and discovery of (new) adventitious agents such as virus microarrays, massively parallel or deep sequencing and broad range PCR combined with mass spectrometry could further contribute to the safety of biological products including vaccines. These new technologies still need to be validated for their intended use, determination of their performance parameters and how they can be applied to the safety of biological medicines. Efforts are ongoing to obtain data for scientific-decision making by regulators and industry regarding the use of the new technologies for evaluation of biological products. This was the focus of the 2013 PDA/FDA meeting on Advanced Technologies for Virus Detection in the Evaluation of Biologicals: Applications and Challenges [67]. Data was presented on the current use of the technologies for investigation of potential contaminants and characterization of cell substrates. Challenges for their routine use were identified plus ongoing group efforts were described. This meeting extended the discussions of the September 19, 2012 FDA Vaccines and Related Biological Products Advisory Committee (VRBPAC) on the use of human tumor cells for vaccine manufacture, which supported the use of the new technologies along with the currently recommended assays for detection of known and unknown viruses in novel cell substrates [68].

6. Proposals for Archiving Vaccine Samples

Comprehensive archiving samples of vaccine batches as well as the cell lines used for production would allow future retrospective analysis of vaccines by new (and presumably)

improved technologies. In addition to the retention of physical samples, in order to investigate the impact of a contamination with an adventitious agent, a system of traceability for the used batches is proposed and should be in place. Retained samples from the seed lot and the cell bank, as well as of raw starting materials, would allow future scientists to determine the source of the contamination and who may have been exposed. In the conduct of clinical trials, samples of patients' sera and peripheral blood mononuclear cells (PMBCs) taken prior to vaccination and at dedicated time points after vaccination should be stored in order to allow for investigation of the potential for human infection with any adventitious agent transmitted by the vaccine.

6.1 Type of Storage

Vaccine samples should be frozen rapidly and stored below −70 °C to enhance retention of the viability of a live viral contaminant. For the purposes of future investigation of adventitious agents in cells, these should similarly be stored below −70 °C, although to retain long-term viability of cells, storage in the vapor phase of liquid nitrogen is required. Samples should be stored in suitable containers but preferably in the original containers to avoid any possibility of contamination being introduced during preparation for storage. A system should be in place for identifying and cataloguing stored samples.

6.2 Length of Sample Archiving

Current US and EU regulations [32, 33] require manufacturers to retain a vaccine sample for one year post expiration of the vaccine (at the temperature that is indicated for the specific vaccine), vaccine ingredients that are used in the process, and 5% of each lot from the Phase I and II clinical trials for two years past the expiry date of the vaccine. However, in order to allow adequate retrospective testing for adventitious agents in vaccines in future years, past experience suggests that samples should be archived ideally for a minimum of 25 years.

6.3 Samples for storage

For long-term archiving purposes it is proposed that for each batch of investigational or developmental (i.e. those used in pre-licensure studies) and commercial (i.e. licensed) vaccine, at least 10 ml of unformulated bulk and at least 10 vials/syringes of the final vaccine should be archived. Consideration has to be given to the quantity of vaccine likely to be required for analysis by any particular technique [32, 33]. This can be assessed for current technologies but is difficult to assess for future technologies; quite simply, the more, the better. Since regulatory laboratories are unlikely to have the resources to enable them to perform such archiving, this would have to be undertaken by the manufacturer of the vaccine. This however would probably require a change to the regulations and is unlikely to be achieved easily. This would not preclude a regulatory laboratory storing samples on an *ad hoc* basis and there may be room for negotiation between a government agency and the manufacturer as to where and by whom samples are archived. If a company were to dissolve, the company would be responsible for the transfer of the samples to a competent regulatory authority for archiving.

6.4 Financial Responsibility

The purpose of this document is to provide technical considerations for guidance and not to determine financial responsibility for the cost of sample archiving. However, it is recognized that the financial burden for appropriate storage of samples above current regulatory requirements would be substantial, possibly even prohibitive. Despite this, the value of archiving material should not be underestimated and attempts should be made to establish a robust archiving system beyond that required by current regulations [32, 33]. Indeed, novel viral vectored vaccines that are “live” and have limited processing (i.e., no viral clearance steps) could be prioritized to follow the proposals provided herein as they could be riskiest to have a contamination.

6.5 Future Needs

As noted earlier, there is a need for guidance on archiving of cells used to propagate virus vaccines and of records that provide full traceability of biological materials used in vaccine manufacture. Another key unresolved issue is the ability to track recipients of a contaminated vaccine accurately. Unlike efficacy, the safety of a vaccine usually cannot be measured directly; relative safety can usually only be inferred indirectly from the relative absence of multiple specific adverse events that have been measured. Either the discovery of an adventitious agent in a vaccine or the occurrence of adverse events in vaccinees can prompt an investigation of the vaccine. In fact, there is an ongoing root cause investigation for the recent identification of *Mycoplasma hyorhinis* in an investigational pox vector vaccine [69]. Adverse events get linked to specific vaccine exposures through epidemiological studies, and the possibility of contamination may be evaluated by laboratory testing with *in vitro* and *in vivo* studies, using conventional methods and new technologies, and through genetic sequencing. Epidemiological studies for determining relative risk are possible, however, only if there are records of who were exposed to the contaminated vaccine and who were not. While progress in developing computerized immunization information systems with tracking of vaccine manufacturer and lot number have been made in the U.S. [13], less progress has been made in the ability to track similar information in vaccinees in other countries [70]. Participation in voluntary centralized vaccination records in Canada has been made available through the launch of a phone app “ImmunizeCA app” in September 2014 [71], whilst in the USA, and effective June 10, 2015, applicants of biological products including vaccines are required to submit Lot distribution reports to the FDA according to amendments in 21CFR600.81 [72].

In addition to sample archiving, other aspects of the 2003 IOM recommendations for a “Vaccine Contamination Prevention and Response Plan’ remain undeveloped, such as “strategies for routine assessment of vaccine for possible contamination; notification of public health officials, health care providers, and the public if contamination occurs; identification of recipients of contaminated vaccines; and surveillance and research to assess health outcomes associated with the contamination”[15]. Given the large proportion of the human population exposed to vaccines annually, the large number of vaccine manufacturers and the diversity of their sourcing, the need for such a plan remains urgent.

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