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Meeting report

Adventitious agents, new technology, and risk assessment, 19–20 May 2011, Baltimore, MD

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ABSTRACT

In May 2011, the International Alliance for Biological Standardization, with the cooperation of WHO, FDA, and NIAID, organized a conference on adventitious agents that might be found in biological products using new technology (http://www.iabs.org/index.php/past-conference-reports/116-baltimore-2011-slides). The implications of such findings on risk assessment also were considered. Topics that were addressed included: a) current routine testing – what are we doing now?; b) recent advances in testing – what tests are being explored/applied?; c) examples of finding agents with "new" techniques; and d) risk assessment, including recent WHO activities. A draft algorithm for risk assessment was discussed in terms of its applicability to a variety of potential new agents and the possibilities for improving it.

1. Introduction

Microbial contaminants have been serious considerations since the very earliest days of manufacturing biological products, and the issue has been addressed with the introduction of a variety of tests over the years. Recent advances in technology have led to more signals of potential contamination.

The objectives of the workshop were to: review traditional and new technology for the detection of microbial agents; consider how progress in microbial agent detection can assist in ensuring the safety of starting materials and final products; and consider how risk assessment can help to address potential safety issues.

2. Testing – current routine tests and recent technological advances

This session provided a framework for what existing tests are in use, their performance characteristics, and emerging new technologies and their potential utility and performance characteristics.

Rebecca Sheets from NIAID provided a historical overview of the tests that are routinely performed to detect adventitious agents in cell substrates and in biological products. These include test methods to detect the following:

- Bacterial and fungal sterility
- Mycoplasmas
- Viruses
  - By infectivity in cell culture *(in vitro)*
  - By infectivity in animals and hens' eggs *(in vivo)*
  - Retroviruses (by polymerase chain reaction (PCR)-based assays for reverse transcriptase (RT) activity and by infectivity in cell cultures)
  - By PCR or other *in vitro* biochemical assays
  - By transmission electron microscopy (TEM)

In addition, strategies exist to minimize the risk of transmissible spongiform encephalopathy (TSE) agents entering into product manufacturing processes and materials. While the assay performance parameters of some of these tests are known (e.g., bacterial

Abbreviations: BPL, beta-propiolactone; BSE, bovine spongiform encephalopathy; PCR, polymerase chain reaction; PERT, product enhanced reverse transcriptase; PCV, porcine circovirus; RT, reverse transcriptase; TEM, transmission electron microscopy; TSE, transmissible spongiform encephalopathy; TTV, Torque Teno virus; vCJD, variant Creutzfeldt-Jakob disease; XMRV, xenotropic murine leukemia virus-related virus.

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and fungal sterility), some of the methods have not been systematically subjected to assay validation, because they have been "grandfathered" into current testing strategies, having been used for decades. Particularly, the *in vivo* and cell culture methods have essentially unknown sensitivity and specificity. A project has been undertaken to begin this systematic assessment. TEM, in particular, is known to be a relatively insensitive method, requiring contamination on the order of 10^6 viruses/mL in order to detect an agent. However, like most of the other methods, the value is that they are broad, general screening methods that do not require knowledge of what the contaminant is in order to detect it. Therefore, in order to consider replacing or refining the traditional methods, new methods will need to have similar abilities – to detect unknown contaminants broadly and generally. The other value of the traditional methods is that most require the ability of the contaminant to grow, meaning that they only detect viable or infectious contaminants, which is the characteristic that is of most concern from a safety point of view. However, a major negative aspect of those tests is that if they do not support the replication of an infectious contaminant, it will not be detected. Newer methods may afford the ability to detect contaminants that may be infectious to humans but not detectable in the traditional test systems. However, most, if not all such new tests are also able to detect evidence for non-infectious contaminants, such as traces of inactivated contaminants.

Carol Marcus-Sekura presented the results of her extensive literature review of bovine and porcine viruses that could have a human host range, and thus, be of concern in the production of human biologicals manufactured from bovine or porcine materials. The currently used test methods for these viruses were implemented to protect veterinary, not human, biologicals. Her review revealed gaps left by the test methods and she recommended means to fill those gaps, which should result in a reconsideration of testing regimes by manufacturers and regulators of human biologicals. In particular, there are potentially >69 bovine viruses in 21 virus families and >52 porcine viruses in 17 virus families that could be of concern to human biologicals production. Most of those agents are likely to be missed by the currently required testing. She noted that due to the high demand of the human biologicals industry for fetal bovine serum, there was evidence of illicit use of calf or adult bovine serum in place of fetal bovine serum, and serum collected from various countries of uncertain or negative BSE status being processed in or relabeled as being from "acceptable" geographies increasing the likelihood of additional viruses that may be of concern being introduced into production processes. The presentation included recommendations to address these gaps which included, but were not limited to, incorporation of new test methodologies that would detect the additional viruses, refinement of existing test methodologies to include additional endpoints, mandatory use of gamma-irradiation to sterilize bovine serum more effectively than can be achieved with heat inactivation, screening of bovine serum for antibodies (which may identify a non-fetal source or contamination, as well as potential interference with the tests), and implementation of a mini-pool concept like that used in human blood screening, which would increase the sensitivity of detection by minimizing dilution of a single contaminated animal's donation.

Rangarajan Sampath of IBIS Biosciences discussed the capabilities of their Biosensor technology that is based on a combination of family-specific PCR amplification, accompanied by mass-spectrometry, to identify amplified contaminants. IBIS has developed PCR primers that detect a wide array of bacterial, mycoplasmal, and viral species. The test method does not require prior knowledge of the specific contaminant and has been used for clinical diagnostics, typing of influenza viruses from clinical specimens, and for investigations into the identity of a novel adventitious agent (a new strain of bluetongue virus) detected by more routine test methods. The utility of the method for routine screening for adventitious agents during the production of biologicals remains to be determined. Because the method has the ability to both detect and identify a contaminant, its potential is great. Like many newer test methods, it cannot determine whether the contaminant is viable or not (i.e., whether it is infectious). Additionally, the method is only semi-quantitative. However, most of the routine test methods are only intended to detect, not quantify a contaminant, so this issue may not be a significant drawback if the method proves suitably sensitive given the sample size that can be tested.

Presenting the microarray capabilities at the Lawrence Livermore National Laboratory, Crystal Jiang explained what microarrays are and their relative cost and speed compared with PCR and sequencing. Microarrays cost a bit more than PCR and take more time, whereas sequencing is more costly and more time-consuming than both. Microarrays also have the capacity to detect known and emerging pathogens. Working from a database of known sequences for ~38,000 viruses and ~3500 bacteria, their microarray contains close to 400,000 probes. When evaluating licensed live viral vaccines, in addition to the endogenous retroviral sequences known to be present in the cell substrates used to produce them, they identified the porcine circovirus contamination of a licensed rotavirus vaccine that also was detected by pyrosequencing in the laboratory of Dr. Eric Delwart. The microarray system can also be used to evaluate clinical specimens to identify infections with results available in 24 h. Like other nucleic acid-based detection technologies, determining whether the identified sequence comes from a viable organism or is residual from an inactivated organism is not possible. However, the method is comprehensive, accurate and has good sensitivity and specificity.

Astrid Ferlinz from Life Technologies discussed microfluidics PCR detection systems. One system is Taqman Array cards, which contain PCR reagents to run 384 wells per card – capable of holding up to 8 samples and running 12 to 384 separate assays depending on how many replicates are run per sample. These assays take about 10 min. In addition, they have Taqman Pathogen Detection Assays capable of detecting specific genes from several viruses or viral families. These screening cards allow triplicates to be run for 16 assays of 8 samples. Their OpenArray system uses nanofluidics in a similar fashion, with the ability to analyze as many as 48 samples per plate. They also offer controls and standard reagents for both nucleic acid and serology detection technologies. These reagents are necessary for assay standardization purposes. Like the other PCR and microarray-based technologies discussed above, this system is subject to the problems of small sample volumes, and thus, uncertain sensitivity. Although many sequences from a virus or viral family may be used, primer mismatch to emerging strains or as yet, unknown or unsequenced family members leave the potential to miss viruses within that family or strains of the virus for which the test is being performed. The presenter primarily focused on the clinical diagnostics use of the system; its utility for routine screening during biologicals production remains unclear. Likewise, it cannot readily distinguish between viable and non-viable agents.

A presentation by David Onions from BioReliance described a new technology that is being used for detection of potential adventitious contaminants in cell substrates, viral seeds, and vaccine products. Massively parallel sequencing, also known as deep sequencing or pyrosequencing, allows hundreds of thousands to millions of nucleic acid sequences to be obtained. Significant bioinformatics are required to analyze the massive amount of data and to compare the sequences obtained to known sequences in order to identify what was detected. This can be done by positive or negative selection against a curated database of known viral