The Implication of Molecular Diagnosis and Good Laboratory Practices in the Reduction of Laboratory Animal Disease: An Approach for Management of Healthcare in Animal Facilities

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Abstract

Diagnosis of diseases is concerned with the identification of accurate and precise causal factors and the direct and indirect relationship between host and organisms. Infectious disease diagnosis in laboratory animals includes traditional techniques like direct examination, culture along with immunological assessments. However, these traditional techniques have limitations of sensitivity; time consumption, and accuracy, thereby molecular approaches can be a modern choice to examine the health of laboratory animals. Modern molecular diagnostic techniques, explore nucleic acids based typing characteristics for identifying the health status and pathogen intensities. Laboratory testing on animal models required expertise with extensive statistical analysis, quality control. Despite considerable improvement, an error arises from laboratory experiments. Controlled and continued quality improvement strategy via regulatory guidelines like good laboratory practices (GLP) would serve as the best tool for reduction of errors and monitoring of laboratory animal health. GLP implication in laboratory animal health care management is a landmark tool for reducing the incidences of disease events. This quality system includes planning, monitoring, recording, archiving and reporting components. This planned system contributes in the generation of precise and accurate results of the experiments conducted on laboratory animals. The present study describes the role of GLP and molecular approach in the management of laboratory animal health.

Keywords: Epstein-Barr virus; Hepatitis virus; Radioallergosorbent test; Ectoparasites; Good Laboratory practices
Introduction

Over the past decades, the molecular diagnostic approach in disease management for laboratory animals have become an active substitute of traditional methodologies. Until the last decade, the diagnostic methods employed in pathogen detection involved natural amplifications in culture, usually consume at least overnight. The replacement of robust methods with laboratory-friendly approached of molecular diagnosis has resulted in shifts: viability is a no longer factor for detection, replication, within a day, limit of detection. Zoonoses and pathogens are life threatening causal components of diseases and mortality in developed countries. Weisbroth, a researcher of laboratory rodent pathogens, has divided last 100 years into three periods. The first, from 1880 to 1950, used to be a period of domestication, where several rodent species used for research experiments with improved animal husbandry. The second period from 1960 to 1985 was the time of gnotobiotic evolution, where cesarean re-derivation was implicated by replacing infected stock with uninfected offsprings. Furthermore, the last third period was from 1980 to 1996, which is known as the period of indigenous murine viruses [1]. Quoting the thought of gentlemen in the following way: At the back of a century, an investigator might said stated that ‘I can’t perform my experiment today, because my animals are dead’; in the mid-century the statement slightly modified to ‘I can’t do my experiment today, as my animals are sick’; but today, the definition has changed completely as ‘I can’t do my experiment, as my animals are antibody positive’. So, the awareness in the management of rodent animal facilities nowadays improved the health and experiment quality. Topical countries are the inhabitant of several potential pathogens that require good and quality management of rodents healthcare. The quality system in animal facilities incorporates good laboratory practices (GLP). In OECD principle GLP is defined as “a quality system concerned with the organizational process and the conditions under which non-clinical health and environment, safety studies are planned, performed, monitored, recorded, archived and reported”. The management of animal healthcare with GLP incorporating the recent diagnostic tool facilitate the quality of experimental results. The present review focused on the implication of recent molecular diagnostic techniques and GLP for the management of the animal facility healthcare environment.

Behavior Observation

Laboratory animal behavior is an important factor that plays a role in the maintenance of the health. It is usually studied in a well-controlled environment that is away from the animal’s home cage. The animal suffers from stress conditions while their handling in the laboratory environments, produce difficulties in interpreting the results of the experiments. Performing tests in a home cage are the better solution for this problem. Treating animal in their home cages is beneficial in several aspects as it will be possible to observe an animal at the consecutive time period; it reduces the transportation of animal to other non-home cage. There are several types of activities that contribute to the stress in laboratory animals that includes; (a) introduction of an animal to open space, (b) handling of the cage, (c) abruptly handling of animal induces an increase in the core body temperature (Tb) [2,3]. This approach limits the stress on the animal and reduces the biasing of the results of the experiments. There is little knowledge about the contribution of the behavior in the stress and distress in laboratory animals. It is the matter of the challenge to the researchers and veterinarians. The daily observation of the behavior of an animal includes posture, hunched posture, hair loss, movement, feed consumption, water consumption. The animal should be examined for pain and distress by experts or trained individual daily. It is critical to predicting the stress and distress in some species and it is well established by research that some species exhibit stress and distress with slight changes in their behavior [4].

Parasitological Monitoring

Laboratory Animals infected by the parasites are not suitable for research purpose. Infections lead to the clinical and pathological changes in animals, especially in the immunodeficient model and prenatal animals. Although infection in immunocompetent animals by parasites also shows subclinical changes and may interfere with the result interpretation of the experiments. Depending upon the nature of the association of parasites with animals, these are classified into; (a) ectoparasites that have an association with animal physical tissues and the (b) endoparasites that reside in the animal gut. The laboratory animal should be diagnosed for both parasites. The frequently routine examination for the ectoparasites includes the physical examination based on the morphology of the parasite. While the endoparasites examination should be done with the fecal analysis for their confirmation in the gut,
especially for pin worm and tapeworm, the perianal tape test in usually performed for pinworms.

Pathology Monitoring

Pathology examination is the clinical diagnosis of disease. It confers four components of disease which includes etiology, pathogenesis of the disease, clinical manifestation, and morphological changes. This is a pioneer tool for the examination of laboratory animals. Interpretation of tissue morphology includes the gross and histo lesions, which help in disease diagnosis and health monitoring of laboratory animals. This is useful to monitor the tissue or organ level changes in the laboratory animals. The techniques are useful in eradicating the examining the disease and their causes that are spread in the colony of laboratory animals. Pathology monitoring includes diagnosis of infectious and noninfectious diseases. With the help of pathology technique application in animal house, it is possible to eradicate the disease spread in the colony of laboratory animals. Nowadays, several advanced techniques have come into existence with the advancement in the research field.

It is the pioneer tool for the evaluation of the laboratory animal health. This tool incorporates the laboratory study of blood serum. Serological reaction is antigen-antibodies based reactions that are used for identification and quantification of antibodies and antigens. This approach relies on the antibodies produced against any infective or diseased conditions of laboratory animals. Infection or disease may be diagnosed by an antibody response to causal organisms. This is the useful approach for diagnosing the microorganisms which are not able to culture and isolate. There are several neither viruses which cannot be isolated nor culture like Epstein - Barr virus and hepatitis virus. There are various types of serological detection of diseases that are efficient for monitoring the laboratory animal health, which is listed as.

Enzyme-linked immunosorbent assay (ELISA)

This method is widely used for monitoring the laboratory animal health around the globe in almost every animal facility. This reaction includes a change in color by the interaction of antibodies in vitro. It is the powerful tool for detection of antigen or antibodies in the serum of laboratory animals.

Advances in Clinical Toxicology

Radioimmunoassay (RIA)

This technique is also depending on antibodies and antigen interaction. It is a very sensitive technique that quantifies antigen like a hormone in the serum with the help of antibodies. It incorporates the use of a radioactive substance, so this technique requires the proper care and performed in the laboratory in the presence of experts. Rast (Radioallergosorbent test) is the precise example of the quantification of allergen in laboratory animals suffering from the allergic disease.

Enzyme-linked immunosorbent spot (ELISPOT)

Cecil Czerkinsky discovered this test in 1983. This serological test is useful for determining the antibodies produced by B cells along with the cytokine response. This test helps in the monitoring of immune response in laboratory animals. This test is both qualitative as well as quantitative test.

Indirect Fluorescence Antibodies (IFA)

This test is used to demonstrate the antibodies present in the serum of the laboratory animal against a specific antigen. This test is based on the fluorescence of the dye labeled antibodies. With the advancement of the research, the techniques also have to be improved.

Hemagglutination inhibition assay (HAI)

This refers to the agglutination of the antigen. It is applicable for the quantification of the specific amount of antigen present in the serum. This test also refers as HI test. It is the sensitive procedure and can detect the soluble antigen presence in the serum samples.

Acute Phase Proteins

These are classes of proteins that includes the C-reactive proteins and other plasma protein rose in the in the patient after the inflammatory response. It is the biological indicator used to monitor the health of the organism. (Claude Bernard’s milieuinterieur). Changes in the acute phase protein in the plasma or serum instruct the inflammatory response in the animal. In one study the C-reactive protein concentration over 100 mgL. represent bacterial infection.

Molecular Biology Testing

Recent landmark progress in proteomics and genomics reshaping our approach to diagnosis. This is another important tool to monitor the health status of the laboratory animals at DNA or Protein level. This tool is
reliable and imparts the valuable information about the animals which is reliable and give results of changes in gene or protein level. Molecular diagnostic methods provide the essential details of health modifiers agents and associated mechanism. There are several molecular biology tools which are listed as

**Specific polymerase chain reaction (PCR)**

Specific PCR is the basic and simple approach for detecting the microbes in the biological samples of laboratory animals. In specific, PCR specific known sequence of primers is designed with tools of NCBI, designed complementary to a known sequence of target DNA that proves to be specifically for a designated class of microbes. As the approach is specific and can be used for direct detection of target microbes responsible for the cause of disease in laboratory animals. This method is widely used in the identification of anti-ds DNA antibody, particularly recognizes the resulting product of amplification.

**Reverse transcription-polymerase chain reaction (RT-PCR)**

Reverse transcription-PCR is the approach for synthesizing the expressed cDNA from RNA by reverse transcriptase firstly, followed by further amplification of a specific cDNA by PCR. This sensitive approach quantifies the mRNA express by pathogens in terms of CDNA. RT-PCR is most widely used in detecting the expression of viruses, the viability of microbes via mRNA.

**Real-time polymerase chain reaction (RT-PCR)**

This is foremost sensitive methods of detecting the pathogenic DNA of microbes or pathogens in laboratory animals that detect, confirm and quantify the PCR products as generated realtime. Recently available fully automated systems, perform the real-time monitoring of fluorescently labeled probes that binds to double stranded DNA in the course of amplifications. This automated and sensitive system not only serves as time savior, but can detect the quantity of DNA in nano concentrations. Thus, careful attention is needed while working on this system with target genes locus [8]. A new study also showed the importance of reload time PCR in the precise diagnosis of disease [9,10].

**Precincts of molecular methods in health care of laboratory animals**

Despite significant rewards of molecular diagnostic approaches, it cannot yet completely replace the other conventional methods for identification of a broad range of infectious disease. The conventional methods due to its inexpensive nature widely employed in the testing of health care of laboratory animals. Recent advances in conventional techniques have resulted in the development of several antigen tests that rapidly requires less than a minute if equipped with a modern automated setup. Unlike bacterial cultures, which can identify a large number of bacteria without initially knowing the responsible organism, all PCR methods except the broad range PCR, can only detect the organism whose DNA is complementary to the target DNA based upon amplifications. Therefore, to identify a similar breadth of the possible culprit organism of laboratory animals causing diseases would require the introduction of simple, inexpensive microarray technologies [8].

**Good Laboratory Practices (GLP)**

The purpose of the principles of GLP is to maintain the quality system and to ensure the good health of laboratory animals. The history of GLP originated in the 1970s during the validation of non-clinical data safety to Food and Drug Administration (FDA). Quality assurance (QA) is a prime component of GLP. GLP can also define the requirement of minimum QA to ensure the integrity of the study thereby authenticating the experimental results. GLP strictly applies to non-clinical studies and testing only, doesn't to clinical studies. Furthermore, GLP assembly depends on the roles and responsibilities of the Study Director. GLP is a quality management system rather than scientific management system [11].

**Conclusion**

Molecular diagnostic techniques have a vital contribution in maintaining good health of laboratory animals. Management of quality experiment depends on the health of laboratory animals. Recent advancement in molecular techniques enables the researchers to ensure quality results in fewer time efforts. Replacement of conventional microbiological approach with molecular approach has proved revolution in the research field. Incorporation of GLP together with molecular tools may contribute to the improvement of animal healthcare in laboratory animals.

**References**


