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Latest diagnostic techniques in rodent pathogens

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Abstract

The use of animal models is critical to the biomedical research. Animals used for biomedical research should be in a state of absolute good health for reliable and reproducible results. It has been reported that infections,environmental factors, genetic factors and interactions of all these may influence the suitability of an animal for research.Sometimes, apparent healthy animals also suffer from latent infections. Majority of these infections are subclinical and may go undetected in gross examination, but clinical symptoms may appear under conditions of stress during experimentation. Also, it has been reported that even subclinical infections in rodents modify or alter research outcome. Many infectious agents affect results in the field of immunology, physiology, reproductive physiology, oncology and many more research areas. Hence, proper and periodic health monitoring programme is important to define the health status of experimental animals. In India, more than 1500 facilities are using laboratory animals for biomedical research. However, majority of them have not adopted the comprehensive health monitoring or disease diagnostic programme due to prohibitive cost of the diagnostic kits. Few facilities in India have adopted international guidelines in health monitoring/disease diagnosis and this includes conventional culture techniques, ELISA and PCR for rapid diagnosis. However, recent techniques such as MFI, Micro ELISA, Microarray and LAMP that have been developed and adopted elsewhere needs to be adopted in India for rapid and accurate diagnosis of pathogens in rodents.

Key words: Rodents; diagnosis; PCR; LAMP; ELISA

Introduction

It has been well established that many rodent pathogens cause sub-clinical infections and affect research the significantly. Health monitoring of experimental animals is essential for more reliable and reproducible research data, and to reduce the risk of transmission of zoonotic infectious agents to personnel handling such animals. Need for sufficient and reliable information about animal health status is gaining more importance since last decade with the rapid development and worldwide exchange of new genetically modified rodents, as well as the globalization of contract research. Timely and accurate diagnosis of infectious disease in rodent colonies is critical to the success of biomedical research. To assess the quality of animals used for scientific research, a proper health monitoring scheme is important to define the pathogen status of both individual animals and the population as a whole. Systematic and scheduled laboratory testing is the most effective way to determine colony status and to prevent or detect influences on experiments. In the 1980s, the US and Japan provided guidelines for both producers and users on the microbiological monitoring of laboratory mice and rats (Allen and Nomura, 1986). These were later revised due to rapid growth of monitoring activities and advances in diagnostics. Hence, more than 100 pathogens of mice and rats were listed

as known and potential agents interfering with biomedical research (Waggie*et al.,* 1994). In Europe, frequent monitoring is recommended only for the most prevalent agents, with less frequent monitoring for the rare agents (Nicklas*et al*., 2002). A number of suggestions for establishing health monitoring programmes have been published in the past few decades (Nicklas 1996; Kunstyr and Nicklas 2000; Gaertner *et al*., 2007).

Conventional methods

Even today, the detection of pathogens by *in vitro* culture method aided with battery of biochemical tests is considered as Gold standard. Culture techniques are most effective during the height of an infection, prior to the administration of antibiotics and prior to the production of an immune response. The main limitations of culture techniques are that not all microorganisms grow well in the media and it can take up to two weeks to culture and identify the infectious agents.

Serological methods

Serological testing for the detection of antibodies to infectious agents is an important component of a comprehensive rodent health monitoring programme. The enzyme-linked immunosorbent assay (ELISA) has been used as a diagnostic tool in various animal facilities to detect the presence of antibodies against particular antigens. Serologic assays rely on the detection of serum antibodies produced during an infection. Antibodies generally are first detectable by 5-7 days post-infection and last for many months. The ELISA is a commonly used serologic test (Kendall and Riley, 1999b). The ELISA is highly sensitive, rapid and inexpensive. However, non-specific cross reactivity between irrelevant antibodies present in the test sera and the antigen used may cause false positive results. This cross-reactivity can be reduced by using highly purified antigens. The specificity of serologic assays is based on the specificity of the antibodies produced for the causative organism.The indirect fluorescent antibody (IFA) assay is also commonly used to detect infectious agent-specific antibodies.The IFA is highly sensitive like ELISA andis rapid and inexpensive. However, interpretation is subjective and is highly dependent on the expertise of the observer (Kendall *et al.,* 1999a). Hemagglutination inhibition (HAI) assays were major serologic testing previously but now its use is very limited (Kendall *et al.,* 1999b). Use of this assay is restricted to viruses, which possess proteins (hemagglutinins) on their surface that bind to red blood cells of specific animal species. HAI tests lack sensitivity, but are highly specific and can be used to differentiate between closely related viruses such as minute virus of mice (MVM) and mouse parvovirus (MPV). Interpretation of HAI test results is highly subjective, which may complicate definitive diagnosis. The microscopic agglutination test (MAT) is the gold standard for sero-diagnosis of leptospirosis because of its unsurpassed diagnostic specificity(Goris and Hartskeerl, 2014).Conventional ELISA tests for diagnosis of diseases are performed on micro-titer plates and it is tedious assay (Dong *et al.*, 2014). A micro fluidic system, which was originated from the concept of miniaturized total analysis system (μTAS) is a promising tool to overcome these problems. A microchip based ELISA (micro ELISA) has also been developed recently by introducing micro beads with immobilized antibodies into a micro channel. This test is currently used in the detection of Foot-and-mouth disease (FMD) virus and further can be adopted to detect other laboratory animal viruses.

Fluorescent methods

Multiplex Fluorescent Immunoassay (MFI) is a sensitive and specific serologic test that allows simultaneous detection of antibodies to multiple viral and bacterial agents in a single reaction well. MFI is a high-throughput assay that offers several advantages over other prevalent assays and is being employed in laboratory animal diagnostics. MFI offers many advantages over ELISA which includes high sensitivity and specificity, better reproducibility, faster throughput of samples, the ability to assay for up to 100 different antigens, multiplexing and most importantly the ability to perform all primary testing by using only 0.2 μl of undiluted serum.Multiplex Fluorescent Immunoassay is based both on bead-based immunoassay and flow cytometry. Each purified antigen or control preparation is covalently linked to one of 100 different types of polystyrene beads, which vary slightly in the intensity of their color. If antibody to a particular antigen is present, it will bind to the antigen on a specific bead and will then be detected by subsequent binding of goat anti-mouse antibody conjugated to a fluorochrome. The reader channels single beads through a dual laser detector which simultaneously determines both the bead type by the internal dye combination and the fluorescent intensity associated with each individual bead(Hsu *et al.*, 2007).

Flow cytometry has evolved as an important tool for providing speedy identification of cell parameters along with great statistical accuracy. Cytometer can handle thousands of cells in few seconds and analyze them individually. Past decade has seen enormous development in the field of cytometry as well as its usage in biomedical research. Flow cytometry has vast application in microbiology too. This includes counting the bacterial cells, bacterial cell cycle analysis and assessment of antibiotic susceptibility of clinical samples, aquatic microbial studies etc. (Steen *et al.,* 1982; Gant*et al.,* 1993; Valdivia and Falklow 1998; Button and Robertson, 2001). Flow cytometric measurements can be made on several different characteristics

of each cell. Such multi-parametric measurements are useful to correlate different characteristics and define sub-populations and distinguish between different cell types. Since measurements are made on single cell, heterogeneity within the population can be detected and quantified (Pils*et al.,* 2006). The flow cytometry measurements of different bacterial colonies were determined in laboratory animals by using scatter pattern as a tool in identifying bacterial species (Rosa *et al.,* unpublished data).

Molecular methods

Molecular methods are commonly being employed to detect rodent pathogens. These are aimed at detecting the nucleic acid (DNA or RNA) genome of infectious agents. The specificity of molecular techniques is based on binding of complementary nucleic acid sequences to each other. The most common molecular methods used to detect infectious agents utilize polymerase chain reaction (PCR) methodology which involves rapid and specific amplification of deoxyribonucleic acid (DNA) (Kendall and Riley, 1999a). PCR is highly sensitive due to the exponential amplification of the template DNA, highly specific due to the specificity of the primers and also rapid. However, minute amounts of contaminating DNA can lead to false positive results and inhibitors of the thermostable polymerase can lead to false negative results. The sensitivity of PCR is its greatest advantage, but it is also one of its greatest disadvantages. Contamination of negative samples with only minute amounts of nucleic acids from a positive sample can result in false positive results. Therefore, strict precautions must be taken to avoid cross-contamination of samples. In general, the sensitivity of viral PCR assays is 1-10 virions, while bacterial PCR assays are capable of detecting as few as 3-10 bacteria (Crompton and Riley, 2001).

Loop mediated isothermal amplification (LAMP) is a powerful innovative gene amplification technique emerging as a simple rapid diagnostic tool for early detection and identification of microbial diseases. PCR-based methods require either high precision instruments for amplification or elaborate methods for detection of the amplified products. The LAMP assay is cost effective and accurate method. In LAMP assay, six different primers specifically designed to recognize eight distinct regions on the target gene are used. The amplification proceeds at a constant temperature using strand displacement reaction.Amplification and detection of a gene can be completed in a single step, by incubating the mixture of samples, primers, DNA polymerase with strand displacement activity and substrates at a constant temperature (about 63°C) (Parida*et al.,* 2008).

A DNA microarray is a collection of microscopic DNA spots attached to a solid surface. DNA microarrays are being used to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. This technology has been employed to investigate the differential gene expression of pathogens, detection and identification of various pathogens, pathogen discovery, antimicrobial resistance monitoring, and strain typing. Microarrays have emerged as potential tools for bacterial detection and identification given their high parallelism in screening for the presence of a wide diversity of genes (Gentry and Jhou, 2006).

Conclusion

Rapid and accurate detection of rodent pathogens are very critical for the effective health monitoring programme. This rapid detection of microorganisms in few hours would certainly benefit the animal facilities in adopting effective, preventive or curative measures in short span of time

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