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AN OVERVIEW OF EXPERIMENTAL RODENT PATHOGENS: HEALTH MONITORING AND LATEST DIAGNOSTIC TECHNIQUES

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ABSTRACT

The system of Rodent health monitoring is critically and crucially dependent upon diagnostics. There are two most important reasons to keep research rodents healthy and free from infections those are; to protect the health and welfare of research rodents and detection and subsequent elimination of disease factors at early stages by using ideal methods can prevent infections from negatively impacting research. Sub clinical infections in rodents modify or alteration in research outcome. In India, over and above 1500 facilities are using laboratory animals for biomedical research. However, majority of them have not implemented the comprehensive disease diagnostic programme or health monitoring due to prohibitive cost of the diagnostic kits. Few facilities in India have implemented international guidelines in health monitoring/disease diagnosis and this includes conventional methods, ELISA and PCR for rapid diagnosis. The expansion of various molecular techniques has developed diagnostic procedures by providing specific diagnosis or detailed characterization of any pathogen or host pathogen interactions. Some of the current immunological and molecular techniques in addition to the conventional ones for the diagnostic technique are discussed in brief. These techniques have been advanced and recognized elsewhere needs to be implemented for rapid and accurate diagnosis of rodent pathogens.

Keywords: Health Monitoring, Diagnostic technique, Rodent pathogen, Diagnosis.

1. INTRODUCTION

Every year more than one million animals are being used for scientific research in India, covering several life science fields like molecular biology, cell biology, bio-chemical and bio-medical research, pharmaceutical science, drug development, veterinary science, food technology and analysis and cosmetic industries etc. To acquire authentic and reproducible results of the research experiments, it is important to have standardized animals with known health and genetic status. The main objective of laboratory animal management is to provide healthy standardized animals for research work [1-3]. The intention of rodent health monitoring programs is to resolve the existence or nonappearance of pathogenic microbes (viruses, bacteria, endoparasites, ectoparasites, protozoa, and fungi) within colonies of laboratory rodents. Because most of the pathogens of laboratory animals do not cause overt clinical disease, identification of these important pathogens depends on a variety of specialized diagnostic tests. Most facilities will not have the capacity to perform such important, but very specific investigations as a service on a routine basis [4]. The health monitoring program of laboratory rodents is predominantly established to identify Pathogens of laboratory rodents cause subclinical infections which might influence the physiological characteristics of the laboratory animals and secure the reliable and reproducible biomedical research data. It is the only reliable basis for rodent pathogen status or health quality assurance and to reduce the risk of transmission of zoonotic infectious agents to personnel handling such animals.The quality of animals used in research has a direct impact on the value of that research. Accordingly, standardized periodic rodent health monitoring, plays vital role in assessing the quality, microbiological status and the suitability of laboratory animals for experiments [5, 6]. Most of the monitoring programmes will primarily focus on infectious agents. It is usually not the aim of a health monitoring programme to define physiological characteristics (e.g. normal values of liver enzymes, CD-antigens, lymphocyte populations, IgG Subclasses for specific mouse strains, tumour prevalence) although these factors might be important

for proper performance of experiments. A number of reports have demonstrated the consequence of microorganisms as factors that may influence animal experiments [7-9]. Established health monitoring helps to avoid preliminary results and enables the implementation of required experiments with a minimum number of animals. Subsequent elimination and recognition of infective factors is consequently means to get improved and more consistent results in animal experiments [4]. Nowadays, extensively used hygienic monitoring programs and hygiene procedures lead to massive improvement of the microbiological eminence of laboratory animals, generating breeding colonies, which are free of pathogens and even free of most opportunistic pathogens [10, 11]. From another view point, the developments in laboratory animal disease control in the following way: At the beginning of new century, an investigator might have said, "I can't do my experiment today because my rats are all dead"; at the midpoint of the current century, an investigator might have said, "I can't do my experiment today because my rats are all sick"; while today, an investigator might say, "I can't do my experiment today because my rats are antibody positive". Confidently, there has been a steady increase in the attentiveness of the varied and generally unwanted effects of natural pathogens in laboratory animals and there have been evergreater efforts to exclude pathogens from research animals. Laboratory animals are at the stage of pathogen free condition which modifies host physiology can valid experimental data be produced and interpreted. When working with rodents in laboratory, researchers and scientists need to confirm that animals are not affected from natural infections [8]. Predominance surveys of murine infectious agents reserved at research institutions in North America, Europe, Japan, Australasia and Taiwan [12-16] over the previous decade have established that infectious agents are still quite predominant in research rodent colonies. The aims of health monitoring have changed in recent decades. In the 1970s clinical disease-often caused by viruses, bacterial pathogens, parasites, and, most frequently, combinations of different agents was not uncommon; testing focused on sick animals and on determining the causes of disease or death in a population. The introduction of re derivation techniques led to the elimination of numerous agents, including, importantly, indigenous murine viruses in rodent colonies (even if they caused only silent infections). In the 1980s, the US and Japan provided guidelines on microbiological monitoring of laboratory rodents for both producers and users [17]. These were later reviewed due to accelerated growth of monitoring activities and advances in diagnostics. Hence, more over 100 pathogens of rodents were listed as known and probable agents interfering with biomedical research [18]. In Europe, regular monitoring is endorsed only for the most dominant agents, with less frequent monitoring for the exceptional agents [19]. Several numbers of suggestions for establishing health monitoring programmes have been reviewed in the past few decades [4, 20, 21]. The health monitoring recommendations for rodents dispensed by the Federation of European Laboratory Science Associations [22] could serve as a model for global recommendation and for international synchronization. The 10th Federation for Laboratory Animal Science Associations [23] symposium in 2007 was the first FELASA symposium at which this question was raised in one talk [24]. At the 2010 FELASA symposium, there were five oral presentations on the influence of the microbiota on laboratory rodents [25] and at the FELASA symposium in 2013 this further increased [23]. International harmonization of HM standards has been recommended along with encourage the 3Rs (i.e., replacement, reduction, and refinement) in utilization of animals [26]. In 1947, Henry Foster established the United States breeding company later identified as Charles River. With the concomitant introduction of the 3Rs (refinement, reduction, replacement) [27] to animal-based research, he initiated the use of cesarean section and barrier defense as a way to enhance rodent models by generating them free of well-defined specific pathogens [28]. Nevertheless, there is the admired intention to keep standardized research animals infectious diseases free, subsequent the basic standard of animal welfare with the 3Rs as its support. On the other hand, researchers aim to bring outcomes that can be reproduced, replicated and finally interpreted to the human situation, following the principle of ensuring scientific value. The latter has recently been laid down in the concept of the 6Rs, which takes both animal welfare and scientific value into consideration [29]. Characteristics central to the success of monitoring rodent colonies for infectious agents consist of knowledge of the pathogenesis and prevalence of the infectious agents; also required are acquaintance with available diagnostic tests and access to specific evidence about the husbandry conditions of individual animal groups. This statistics will ultimately dictate which agents to review, how frequently testing are

needed, and which method of testing to employ. This article focuses on available testing methodologies, and general issues in laboratory rodent animal health monitoring and the frequency of testing. In addition, the reader will obtain current information on the prevalence of infectious agents as an indicator of the potential risk of these infectious agents invading a research rodent colony.

2. HISTORICAL PERSPECTIVE OF LABORATORY RODENTS HEALTH MONITORING

During the last century, tremendous development has been made on the subject of animal housing and gnotobiotic derivation processes, focusing at developing the microbiological quality of mice and rats bred in laboratory research facilities. Revaluating the past 140 years, many rodent species were transported indoors to become consequential used research subjects. Their relocation to an indoor environs, together with the many developments made in laboratory animal care, resulted in a great reduction in the collection and predominance of pathogens, particularly those requiring transitional hosts or other vectors and those commonlyconcomitant with an outdoor environment. Theasepticcondition of animals used for scientific determinations evolved from aninitial phase of domestication (1880-1950), in this phase several rodent species were transported indoors to become much used research subjects. Their relocation to an indoor environment, together with the many developments made in laboratory animal care, resulted in a rapid reduction in the range and prevalence of pathogens. The second phase of gnotobiotic derivation (1960 to 1985) was considered by the development of systematized laboratory animal science and medicine, resulting in part from a documented need to address the enduring problem of laboratory animal diseases. Developments of this period in animal husbandry and facility operation also contributed to this end.In which breeding stocks were recognised with animals obviously infected with diverse pathogens, over extensive gnotobiotic rederivation processes. Weisbroth has pronounced the third period, (1980-1996) to a period where indigenous viruses and other specified pathogens were eliminated from laboratory rodent colonies. In this period, additional pathogens dropped from the scene or were found less and less often. This approximately divided timeline, previously chronicled by Steven H. Weisbroth and David G. Baker in the late 20th century [5, 6], can be extended to the most recent and still on-going situation, the phase of an isolated animal husbandry with limited microbial exposure. Though this development follows an intrinsic logical path, it turns out that the current situation also brings its own challenges.

Table 1: Rodent pathogens and research impact

Agent	Host	Adverse Effects/Research Impact
Mouse Hepatitis Virus (MHV)	Mouse	Immunosupression, compromised CNS and gastrointestinal tract, unexplained deaths
Sendai Virus	Mouse, rat, hamster, Guinea pig	Immunosuppression, neonatal and adult deaths, respiratory lesions, interruption in breeding
Minute Virus of Mice and Mouse Parvovirus (MVM, MPV)	Mouse	Immunosuppression, low ascites production, impact on lymphocyte cultures
Rat Parvovirus, Kilham's rat virus, Rat Minute Virus and Toolan's rat virus (RPV, KRV, RMV, H-1)	Rat	Immunosuppression, impact on lymphocyte cultures and oncology studies
Theiler's murine encephalomylelitis virus (TMEV, GDVII)	Mouse, Rat	Immunologic and CNS impact
Enzootic Diarrhea of Infant Mice (EDIM)	Mouse	High mortality in young mice less than 2 wks old, diarrhea, alters intestinal absorption
Mouse Adeno Virus (MAD, MadV-1, K-87)	Mouse, Rat	Kidney lesions, causes wasting in nude mice
Pneumonia Virus of Mice (PVM)	Mouse, rat, hamster, gerbil, Guinea pig	Pulmonary interference, wasting disease in immunodeficient animals
Helicobacter sp.	Mouse, rat, gerbil	Inflammatory response, gut and liver impact in susceptible strains
Mycoplasma pulmonis	Mouse, rat	Respiratory issues, immunosuppression, animals appear clinically ill
Pasteurellapneumotropica	Mouse, rat, hamster, gerbil, Guinea pig	Reproductive issues and low producing breeders, Respiratory, eye, genital tract and skin infections
Pinworms (Syphacia sp. and Aspicularis sp.)	Mouse, rat, hamster, gerbil	Marker of inadequate biosecurity, rectal prolapse, poor condition, rough hair coats and reduced growth rates

Source: (Niemi and Niemi, 2013)

The governments of the United States and Japan have long recognized the consequence of scientific cooperation and the teamwork that it achieves. A joint program well-established nearly two decades ago by these governments has produced new projects that have facilitated to advance techniques and provide standards for biomedical research around the world. The National Institutes of Health and the Japanese Central Institute for Experimental Animals, under the terms of this program, have long nurtured collaborative, information-exchange activities. The persistence of many leaders-including Drs. Nomura, Kagiuama, Held, and Allen as well as other important contributors-has enormously heightened the genetic and microbiologic integrity of laboratory rodent colonies, not only in the United States and Japan, but worldwide. Advanced microbiologic monitoring for major infectious agents and improved diagnostic techniques for diseases now safeguard our valuable but fragile resource investments, including specific pathogen-free animals.

Infectious diseases of rodents are important to the scientific community because they can introduce unwanted variables that can alter experimental outcomes [9]. Suitable diagnostic assay selection for transmissible diseases depends on multiple parameters including clinical presentation and endemic pathogens known to circulate within a specific geographic region. Rapid point-of-care PCR [30, 31] and lateral flow immunoassays [32, 33] as well as more complex PCR (34-35) and laboratory based antigen capture ELISAs [36, 37] can generate a clinically actionable diagnosis in rodent health monitoring. These assays are sensitive, rapid, and relatively inexpensive, making this testing approach ideal for initial diagnostic testing.

3. OUTSTANDING DIAGNOSTIC METHODS FOR INFECTIOUS MEDIATORS OF RODENTS

3.1. Conventional methods

Even so, the detection of pathogens by *in vitro* culture method assisted with battery of biochemical tests is considered as Gold standard. Culture techniques are most effective throughout the elevation 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.

Table 2: commonly used testing methodologies for mouse and rat pathogens

Agent (species)	Species[a]	Primary testing Methodology (sample tested)	Confirmatory Testing methodology
Viruses			
Ectromelia	M	Serology (serum)	PCR, Histology
Hantaan (HTN)	R	Serology (serum)	PCR
K virus	M	Serology (serum)	PCR
Lymphocytic choriomeningitis virus (LCMV)	M,R	Serology (serum)	PCR
Lactate dehydrogenase-elevating virus (LDEV)	M	Serology (serum)	PCR
Mouse adenovirus 1 (MAD 1)	M,R	Serology (serum)	PCR
Mouse adenovirus 2 (MAD 2)	M	Serology (serum)	PCR, Histology
Mouse cytomegalovirus (MCMV)	M	Serology (serum)	PCR, Histology
Mouse hepatitis virus (MHV)	M	Serology (serum)	PCR, Histology
Mice minute virus (MMV)	M	Serology (serum)	PCR
Mouse parvovirus (MPV)	M	Serology (serum)	PCR
Mouse rotavirus (MRV)	M	Serology (serum)	PCR, Histology
Mouse thymic virus (MTV)	M	Serology (serum)	PCR
Pneumonia virus of mice (PVM)	M,R	Serology (serum)	PCR
Polyoma virus (polyoma)	M	Serology (serum)	PCR
Rat coronavirus (RCV)	R	Serology (serum)	PCR, Histology
Rat parvoviruses (rat parvos)	R	Serology (serum)	PCR
Reovirus type 3 (REO 3)	M,R	Serology (serum)	PCR
Sendai virus (Sendai)	M,R	Serology (serum)	PCR
Theiler's murine encephalomyelitis virus (TMEV)	M,R	Serology (serum)	PCR

Bacteria			
Cilia-associated respiratory	M,R	Serology(serum)/PCR (trachea)	Histology
(CAR) *bacillus Citrobacter rodentium*	M	Culture (fecal contents)	PCR, Histology
Corynebacterium kutscheri	M,R	Culture (NP)[b]	PCR
Helicobacter spp. (any)	M,R	PCR (feces)	Culture
Helicobacter hepaticus	M,R	PCR (feces)	Culture
Helicobacter bilis	M,R	PCR (feces)	Culture
Helicobacter typhlonius	M,R	PCR (feces)	Culture
Helicobacter rodentium	M,R	PCR (feces)	Culture
Helicobacter sp. Unidentified	M,R	PCR (feces)	Culture
Mycoplasma pulmonis	M,R	Serology (serum)/PCR (NP)	Culture
Pasteurella pneumotropica	M,R	Culture (NP)	PCR
Proteus mirabilis	M	Culture (fecal contents)	PCR
Pseudomonas aeruginosa	M	Culture (fecal contents)	PCR
Salmonella spp.	M,R	Culture(cecal contents)	PCR
Clostridium piliforme	M,R	ELISA (serum)	PCR, Histology
Parasites			
Aspiculuris tetraptera	M	Direct exam (colon contents)	-
		Direct exam (fecal floatation)	-
Myobia musculi	M	Direct exam (pelage)	-
Myocoptes musculinus	M	Direct exam (pelage)	-
Radfordia affinis	M,R	Direct exam (pelage)	-
Radfordia ensifera	M	Direct exam (pelage)	-
Rodentolepis nana	M,R	Direct exam (small intestine)	-
Syphacia obvelata	M	Direct exam (fecal contents)	-
		Direct exam (perianal tape test)	-
Syphacia muris	R	Direct exam (fecal contents)	-
		Direct exam (perianal tape test)	-
Fungus			
Pneumocystis cariniic	M,R	PCR (lung)	Histology
Protozoan			
Encephalitozooan cuniculi	M,R	ELISA (serum)	PCR, Histology

[a]*M, Mouse; R, rat.* [b]*NP, nasopharynx.* [c]*Monitored only in immunodeficient mice and rats.*
Source: (Livingston et al., 2003)

3.2. Serological methods

Serological testing for the recognition of antibodies to infectious agents is an important constituent of a comprehensive rodent health monitoring programme. The enzymelinked immunosorbent assay (ELISA) is highly sensitive and accurate immunodiagnostic technique. It has been used as a diagnostic tool in various animal facilities to detect the presence of antibodies against particular antigens. Serologic assays depend on the detection of serum antibodies produced during an infection. The ELISA is a generally used serologic test [38-40]. ELISA method was established in 1971 [41] and It is an alternative to radioimmunoassay methods. Based on enzyme-tagged immunoreagents these methods (EIA/ELISA) have prominent advantages including the long lifespan of the reagents used, being free of radiation risks accompanying with waste elements, facilitating the analyses of multiple samples in a short period. The specificity of serologic assays is established on the specificity of the antibodies formed for the causative organism. The indirect fluorescent antibody (IFA) assay is also frequently used to distinguish infectious agent-specific antibodies. The IFA is highly sensitive like ELISA and is rapid and inexpensive. However, interpretation is subjective and is highly reliant on the expertise of the observer. Hemagglutination inhibition (HAI) evaluation was one of the major serologic testing previously but present its

use is very limited [40]. Use of this assay is delimited to viruses, which possess proteins (hemagglutinins) on their surface that bind to red blood cells of particular animal species. HAI tests are deficiency sensitivity, but are highly specific and can be used to differentiate between closely associated viruses such as minute virus of mice (MVM) and mouse parvovirus (MPV). The microscopic agglutination test (MAT) is the best standard for serodiagnosis of leptospirosis for the reason that of its unrivalled diagnostic specificity [42]. Conventional ELISA tests for identification of infections are performed on micro-titer plates and it is exhausting assay [43]. 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.

3.2.1. Limitations of ELISA method

In direct method limited sensitivity and signal amplification step was absent. This assay is time-consuming, each assay target needs a specific conjugated primary antibody which can also makes the assay relatively expensive and attachment of the enzyme to the antibody may limit the spatial availability of sites on the antibody that should bind to the antigen. In Indirect method, it has more complex workflow and an additional incubation step is required for the secondary antibody. It is potential for cross-reactivity, nonspecific signal due to a cross-reaction with the secondary antibody.

3.3. Fluorescent methods

Multiplex Fluorescent Immunoassay (MFI) is a sensitive and specific serologic test that consents simultaneous recognition of antibodies to multiple viral and bacterial agents in a single reaction well. MFI is a high performance method that offers several advantages over other established assays and is being engaged in laboratory animal diagnostics. MFI endeavors many benefits over ELISA which contains high sensitivity and particularity, improved reproducibility, faster throughput of samples, the capability to assay for up to 100 different antigens, multiplexing and out most importantly the ability to perform all primary testing by using only 0.2µl of undiluted serum. In MFI, microspheres provinces as the solid phase to bind protein antigens from various viral and bacterial disease agents, and two lasers distinguish the presence of serum antibodies to these antigens. Multiplex Fluorescent Immunoassay is established both on bead based immunoassay and flow cytometry. Each purified antigen or control research is covalently connected to one of 100 different types of polystyrene beads, which differ slightly in the strength of their color. If antibody to a specific antigen is present, it will bind to the antigen on a particular bead and will then be distinguished by subsequent binding of goat anti-mouse antibody conjugated to a fluorochrome. The reader routes single beads over a dual laser detector which concurrently determines both the bead type by the internal dye consolidation and the fluorescent concentration associated with each individual bead [44].

3.3.1. Limitations of Multiplex Fluorescent Immunoassay

MFI has no major imperfection compared with ELISA. The limitations it presents are characteristic of other serologic estimations: (i) false positives may exist with binding of nonspecific antibody (ii) immuno-compromised animals which do not produce an antibody reaction cannot be assayed by MFI (iii) recognition of antibodies to pathogens specifies previous exposure and not essentially active infection.

3.3.2. Flow cytometry

Flowcytometry has evolved as an important tool for providing quick identification of cell parameters along with great statistical accuracy. Flow cytometry uses light scatter based on laser technology and fluorescence possessions to identify, count and assess function in selected subcategory of cells. The exclusive light scatter properties of a specific cell type can be used to discriminate and identify subcategories of cells, making this method a crucial part in the laser-based clinical hematology analyzers. [45, 46]. Cytometer can handle several numbers of cells in fraction of seconds and analyze them individually. Past decade has seen rapid improvement in the field of cytometry in addition to 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. [47-50]. Flow cytometric measurements can be made on numerous different characteristics of every cell. Such multi parametric quantities are useful to associate different characteristics and define subpopulations and distinguish between different cell types. Since measurements are

made on single cell, heterogeneity within the population can be detected and quantified [51]. 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).

3.3.3. Limitations of flow cytometry

It method is very expensive and sophisticated instrument it requires management by a highly trained specialist and continuing maintenance by service engineers. (ii) Complex instruments are prone to problems with the microfluidics system (blockages) and also require warm-up, laser calibration and cleaning for each use. Needs single cell particle, tissue structure is lost, little information on intracellular distributions.

3.4. Molecular methods for the detection of infectious pathogens in rodents

Molecular methods are frequently being employed to detect rodent pathogens. These are designed at recognized 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.

Over the last decades, the use of PCR and, in the case of RNA viruses, Reverse Transcription (RT)-PCR techniques have been broadly validated as a valuable alternative to traditional cultural, microscopic and serological pathogen detection methods. As PCR testing is based on the molecular detection of specific genetic sequences of the infectious agents, this method can be used to detect even very small amounts of nucleic acids in various sample types, which not only enhances the diagnostic sensitivity but also facilitates the use of environmental sample material as an alternative to direct animal testing [52]. 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) [40]. 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 [53].

3.4.1. Real time qPCR

Real time PCR is the latest improvement in the standard PCR technique that enables rapid and specific diagnosis of disease outbreaks. Real time PCR depend upon less manipulation, is additional rapid and specific than conventional PCR technologies, has a closed-tube format therefore decreasing risk of cross-contamination, is extremely sensitive and particular, thus embracing qualitative proficiency, and contributes quantitative evidence. Revealing of positive samples is dependent on the quantity of fluorescence released during amplification. It can be used to evaluate DNA or RNA content in a given sample. The thermo cycler (PCR) is also used expansively for the genotyping and phylogenetic analysis of rodent pathogens. In many cases, the real-time PCR assays have proved to be more sensitive than existing reference methods [54, 55].

3.4.2. Diagnosis by DNA probes technique

In DNA probe hybridization the DNA, derivational from sample distrusted of containing a pathogen (the 'unknown'), connect with extremely characterized DNA derived in development from a pathogen of interest (the 'known' DNA). In conventional DNA probing the unknown DNA (or RNA), the objective, is immobilsed on a solid surface e.g. a filter; and the known DNA which is applied to the target, is in the liquid phase. The bound probe can be identified by addition of specific molecules connected to an enzyme that generate colour or light (chemiluminescence). Detection of pathogen by this method is limited by the number of probes used.

3.4.2.1. Limitations of DNA probe method

The limitations of DNA probe assays include: the use of isotopic detection methods for optimum sensitivity, expense of current reagents, limited availability of many probes, limited diagnostic sensitivity of current assays, slow turnaround time for some assay formats, lack of technical expertise in most diagnostic laboratories, and the requirement for antimicrobial susceptibility testing (requires culture).

3.5. PCR-RFLP method

PCR-RFLP is amendment of the basic RFLP method whereby the polymerase chain reaction (PCR) is assimilated as a preliminary step. The PCR technique is used to amplify an exact area of the genome (known variable sequence among pathogens), which then assists as the template DNA for the RFLP technique. This new combination (PCR-RFLP) offers a much greater sensitivity for the identification of pathogens and is especially useful when the pathogen occurs in small numbers or is difficult to culture.

3.6. Loop mediated isothermal amplification (LAMP) assay technique

InLAMP assay technique, six different primers individually designed to identify eight different regions on the target gene are used. The amplification continues at a constant temperature using strand transposition reaction. Amplification and revealing of a gene can be accomplished in a single step, by incubating the combination of samples, primers, DNA polymerase with strand displacement activity and substrates at a consistent temperature (about 63°C) [53]. Loop mediated isothermal amplification (LAMP) is an influential innovative gene amplification procedure emerging as a simple rapid diagnostic tool for early recognition and detection of microbial diseases. PCR established methods require either high precision instruments for amplification or complicated methods for recognition of the amplified products. The LAMP assay method is cost effective and authentic method.

3.6.1. Limitations

Majorly LAMP technique was used as a diagnostic or detection technique but not useful for cloning purposes. Compared to PCR it is less versatile than PCR. In this method major constraint is proper designing of primer [56]. Multiplexing approaches for LAMP are less developed than for PCR.

3.7. DNA microarray method

A DNA microarray technique is an assortment of microscopic DNA spots attached to a solid surface. DNA microarrays are being used to quantify the expression levels of large quantities of genes concurrently 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 appeared as potential tools for bacterial identification and detection given their high correspondence in screening for the occurrence of a wide diversity of genes [57].

3.7.1. Limitations of DNA microarray method

Hybridization-based approaches are high throughput and relatively inexpensive, but have several limitations which include: Reliance upon majorly existing knowledge about the genome sequence. High background levels owing to cross hybridization limited dynamic range of detection owing to both background and saturation signals. Comparing expression levels across different experiments is often difficult and can require complicated normalisation methods [58].

4. CONCLUSION

However there is no single diagnostic testing plan that can be applied to all rodent colonies, one can develop individual testing programs by assessing the risk posed by the various infectious agents (i.e., risk to research, risk to personnel, and risk of exposure of the colony to the infectious agents). Rapid and meticulous detection of rodent pathogens are very discriminating for the effective health monitoring programme. It is impartant to say that the current lack of rapid diagnostic tests for priority pathogens is addressed, to ensure that future outbreaks can be more effectively contained.

Declaration of Competing Interest

The authors declare no conflict of interest.

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