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3Rs expression in quality control paradigms of human vaccines

Abstract

Communicable diseases remain the leading cause of mortality worldwide in past. Children and adolescent were the most affected individuals. However, the development of vaccine played a vital role in decreasing mortality and increasing life expectancy. Currently most of vaccines are based on utilizing animal pathogens and use of animals. This use of animal in quality testing of vaccines is inevitable. Over the past century, concern about animals' interests was limited to ensure that animals be treated humanely and not subjected to unnecessary sacrifice and sufferings. However, with the rise of 3Rs concept the global scenario for use of animals is being changed a little. The current review addresses the refinement, reduction and replacement aspects of animal use in vaccine testing. It also dialogues about challenges to implement 3Rs and gadget this key concept for effective quality testing.The acceptability and implementation of 3Rs concept is based on good manufacturing practices, good in-process quality control and validated procedures and processes. It is easier for new vaccines to adopt this concept. However, several difficulties are still experienced in theimplementation of 3Rs principles for vaccine potency assays. Thus, insistent exploration is obligatory by bothhealth industries and regulatory agencies for the implementation and validation of robust 3Rs approaches around the globe.

Keywords: vaccination, quality control, 3Rs, potency testing

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Introduction

Communicable diseases remain the leading cause of mortality worldwide in past. Children and adolescent were the most affected individuals. However, the development of vaccine played a vital role in decreasing mortality and increasing life expectancy. Vaccines prevented 178 million cases from 1888 to 1924 in United States only.[1] Similarly, 300 million people died of smallpox in the 20th century but no one dies today due to vaccination.[2] Worldwide life expectancy has also been increased.[3] Vaccines are being developed using the principles set by Louis Pasteur since more than 100 years.[4] In last few decades, several technologies have been industrialized to develop vaccines.[5]

The current review addresses the refinement, reduction and replacement aspects of animal use in vaccine testing.It also dialogues about challenges to implement 3Rs and gadget this key concept for effective quality testing.

The vaccines spectrum

Vaccines have saved more lives than any other medicine or medical product around the globe. It has been proved very effective tool of immunization in modern medicine.[6] There are many types of vaccines (Table 1) which are categorized by the antigen used in their preparation.[7]

Quality control testing for vaccines

Biological products are different in comparison to other pharmaceutical products; same is the case with vaccines. They are being derived from activemicroorganisms. However, their composition is complicated to be explained in perspective of chemical of physical means. Moreover, the intrinsic diversity of microorganisms and the potential for contamination of materials use in vaccine production requires special attention for quality control of vaccines.[8] Generalized tests of vaccines for human use include pH, Adjuvant, Aluminium, Calcium, Free Formaldehyde, Phenol, Water, Extractable volume and Bacterial endotoxins.[9] Typically, individual vaccine may contain the following tests;[9]

a. Residual pertussis toxin for vaccines (comprising acellular pertussis factor)
b. Residual infectious virus
c. Sterility test (for live vaccines)
d. Pyrogens/Bacterial endotoxins
e. Total protein content (where relevant)
f. Free saccharide (for conjugate vaccines)
g. Potency test
h. Distribution of molecular size/ molecular weight (for polysaccharide vaccines)
i. Ovalbumin content (where a vaccine is produced in eggs)
j. Bovine serum albumin (where a vaccine is produced in cell cultures)
k. Host cell and vector DNA
l. Host cell protein
m. Residual reagents
n. Vesicle size (virosomal vaccines)

Use of animals in quality control

Research on infections and infestations in veterinarycontributed a significant part in the advancement of vaccines for human use

over past century. Currently most of vaccines are based on utilizing animal pathogens.[10] Likewise, use of animals in quality testing of vaccines has become an important tool. Animals are used in vaccine development and testing to evaluatesafety of vaccine; defense against an infection or illness; decreasein clinical signs &symptoms;death of pathogen; commencement and extent of immunity; category of immune response; routes of vaccine administration; and evaluation of particular immune compartments.[11] Rats, mice[12] or guinea pigs are used in potency tests for vaccines. There is a huge diversity of potency tests for vaccines especially those labelled as bacterial. Code of Federal Regulations specified few standards for vaccines like cholera, typhoid, pertussis, anthrax and BCG vaccines while others like tetanus, plague and diphtheria vaccines follow minimum requirements. On contrary, acellular pertussis, polysaccharide conjugate and live oral typhoid are analyzed according to adapted standards.[13] With the diversity of potency testing, large numbers of animals are used in testing procedures.There are no exact figures available for the use of laboratory animal in quality testing of vaccines. However, there is an estimate of 10% laboratory animals being used in biomedical research and testing. It includes more than one million rodents and guinea pigs only in European countries.[14] Various approaches were adopted to reduce number of animals in animal testing. However, these approaches were based on a change in experimental design, a change based on statistical review and changes resulting from harmonization of test requirements.[15] These approaches accompanied the concept of 3Rs.

Table 1 Types of vaccines with examples

Class	Type	Nature	Example
I	Live attenuated vaccines	contain whole bacteria or viruses which have been "weakened" so that they create a protective immune response but do not cause disease in healthy people	Rotavirus vaccine, MMR vaccine, Nasal flu vaccine, Shingles vaccine, Chickenpox vaccine, BCG vaccine against TB, Yellow fever vaccine, Oral typhoid vaccine (not the injected vaccine)
II	Inactivated vaccines	contain whole bacteria or viruses which have been killed, or small parts of bacteria or viruses, such as proteins or sugars, which cannot cause disease	
IIA	Whole killed' vaccines	contain whole killed viruses	Inactivated polio vaccine or IPV, Some inactivated flu vaccines which are described as 'split virion', Hepatitis A vaccine, Rabies vaccine, Japanese encephalitis vaccine
IIB	Subunit vaccines (sometimes called 'acellular')	do not contain any whole bacteria or viruses at all. ('Acellular' means 'not containing any whole cells'.) Instead contain polysaccharides (sugars) or proteins from the surface of bacteria or viruses. These polysaccharides or proteins are the parts that our immune system recognizes as 'foreign', and they are referred to as antigens	
IIB-1	Toxoid vaccines	Some bacteria release toxins (poisonous proteins) when they attack the body. The immune system recognizes these toxins in the same way that it recognizes polysaccharides or proteins on the surface of the bacteria. Some vaccines are made with inactivated versions of these toxins. They are called 'toxoids' because they look like toxins but are not poisonous	Diphtheria vaccine, Tetanus vaccine, Pertussis (whooping cough) vaccine
IIB-2	Conjugate vaccines	In most conjugate vaccines, the polysaccharide is attached to diphtheria or tetanus toxoid protein. The immune system recognizes these proteins very easily and this helps to generate a stronger immune response to the polysaccharide	Hib vaccine, MenC vaccine, PCV
IIB-3	Recombinant vaccines	made using bacterial or yeast cells to manufacture the vaccine. A small piece of DNA is taken from the virus or bacterium against which we want to protect. This is inserted into other cells to make them produce large quantities of active ingredient for the vaccine (usually just a single protein or sugar)	Hepatitis B vaccine, MenB vaccine, inactivated flu vaccines described as 'surface antigen', PPV, Injected typhoid vaccine (a polysaccharide vaccine)

3Rs concept in vaccine quality control

WilliamRussel and Rex Burch formulated the principles for humane technique firstly in 1959.[16] A milestone was achieved as 3Rs concept in quality control of vaccines in an international forum in Londonin the year 1985. This concept of 3Rs can be translated in the following context:

a. **Replacement** means implementing methods which avoid or replace the use of animals
b. **Reduction** means changing the test design in order to minimize the number of animals per experiment
c. **Refinement** means moving to methods that minimize suffering and improve animal welfare (e.g. replacing challenge tests by immunogenicity assays)

Europe converted this concept into a legal requirement and documented it in European medicines agency guidelines 1997.[17] It was followed by a directive published in 2001 for both veterinary

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and medicinal products.[18] However, the main ordinance was issued in June, 2010 on safe use of animals for scientific research and testing.[19]

Prominence of 3Rs in vaccinology

The development of robust assay in vaccine demands the mechanism for induction of safe immune response and action of pathogen or pathogenic entity in causing disease.Furthermore,this assay development necessitates the insight of virulence factors that exert theirpathogenic effects. Development of assays considering these principles will result in complete replacements of animal models. However, lack of scientific knowledge at presentlimits the development of such mechanism based assays.Currently, the concept of vaccine quality control is being shift from classical model to consistency approach.[20] Thus, the foremost attention should endure in monitoring of consistency rather than to establish the factual efficacy of a vaccine.[21] Four tiers are being used to implement and justify the concept of 3Rs in quality control of vaccines (Figure 1). However, Figure 2 shows few examples of 3Rs for human vaccines potency testing.

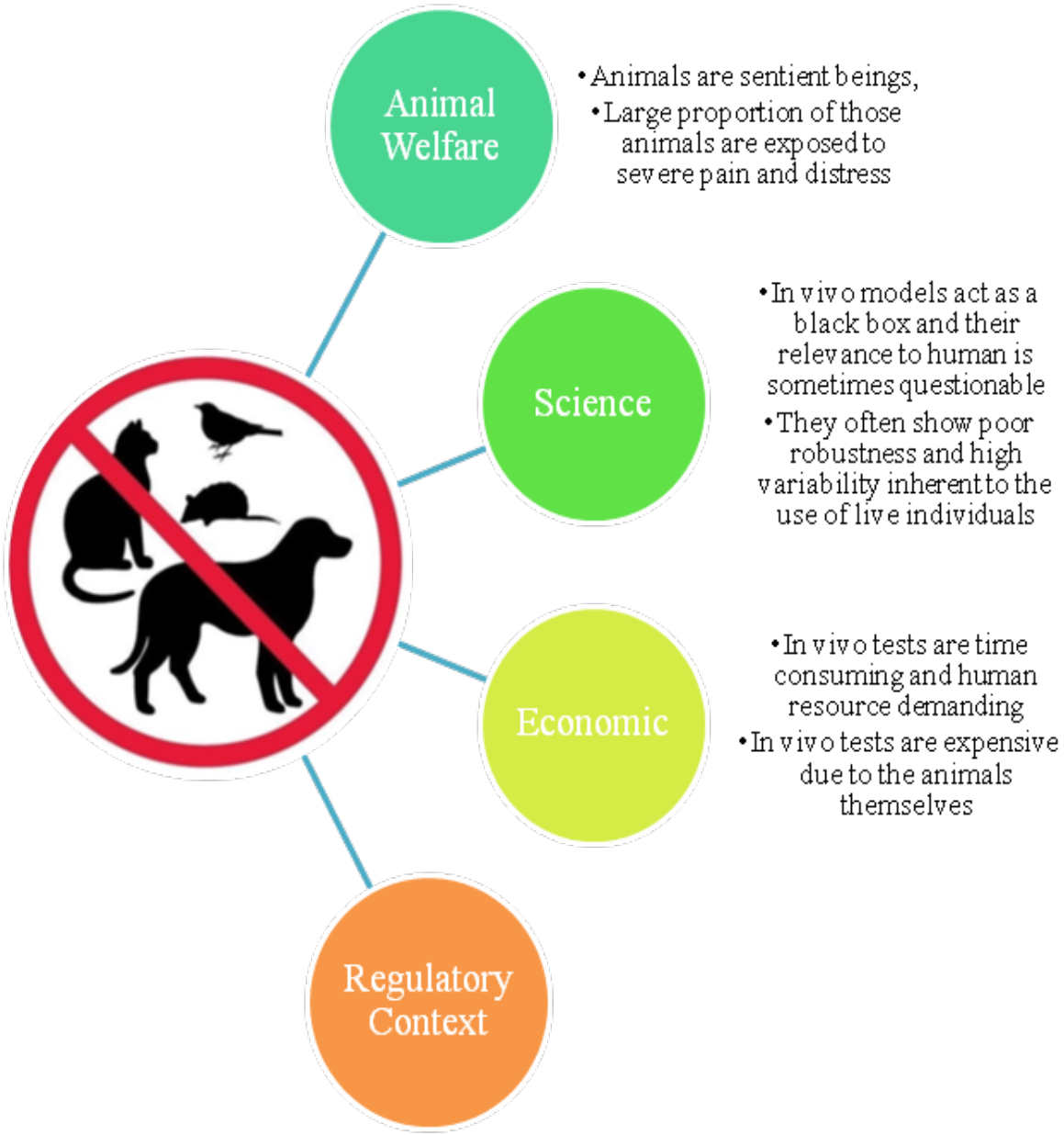

Figure 1 Four tiers of 3Rs.

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Vaccines	3Rs Expression	References
Acellular pertussis	Traditional: The histamine sensitization test Alternate: Combination of enzyme coupled-HPLC (E-HPLC) & carbohydrate binding assays	[22]
Acellular pertussis Component	Traditional: Multiple-dilution mouse serology Alternate: Immunization (mice) and ELISA	[23]
Whole cell pertussis	Traditional: Lethal Challenge potency test Alternate: Evaluation phase and validation phase with humane endpoints	[24, 25]
Anthrax	Traditional: In-vivo mouse immunogenicity test Alternate: Toxin neutralization assay	[26]
Cholera	Traditional: Multi-dilution vaccination & serology Alternate: ELISA	[27]
Diphtheria	Traditional: Lethal/intradermal challenge test, Residual toxicity in diphtheria Alternate: ELISA, Vero cell assay	[28-30]
Diphtheria component	Traditional: Guinea pig lethal challenge test Alternate: Erythema score following intradermal challenge	[31, 32]
Haemophilus type B Conjugate	Traditional: Multi-dilution vaccination & serology Alternate: High throughput SBA for anti-Hib antibodies	[33]
HBsAg	Traditional: In-vivo method Alternate: In-vitro method for HBsAg content using Auszyme EIA kit	[34]
Hepatitis A	Traditional: Mouse serology Alternate: Antigen quantification	[35]
Hepatitis B	Traditional: Potency test Alternate: Serological antigen quantification	[35]

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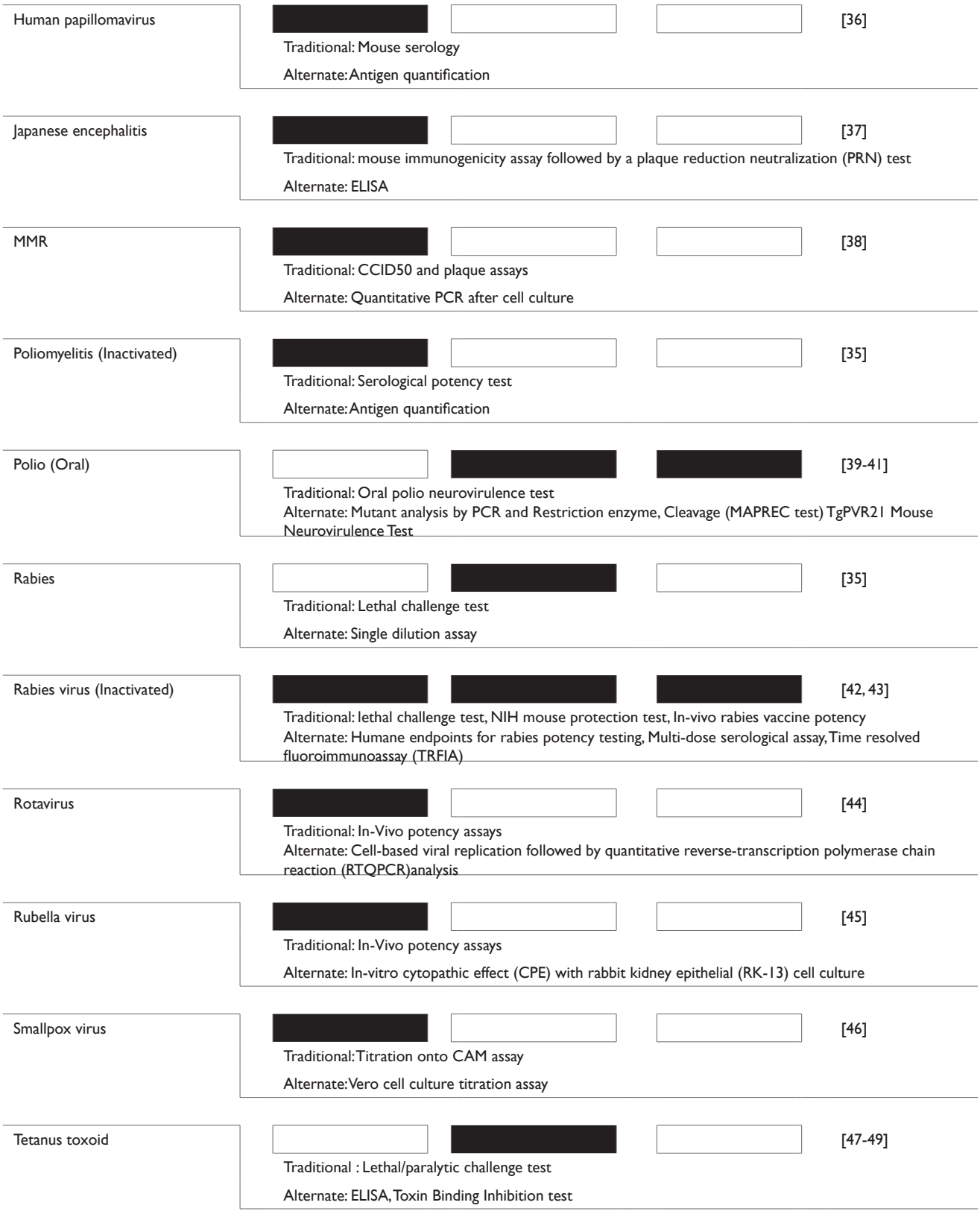

Figure 2 Matrix for 3Rs expression of potency testing (human vaccines).

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Challenges to implementing 3Rs

World health organisation directed to national quality control laboratories to apply the concept of 3Rs. However, there are two main challenges to implementing 3Rs. First is scientific and second is regulatory. Scientific challenge involves the inherent variability of in vivo assays, validation issues of *in vivo* assays as per ICH guidelines[50] and the attributes of product quality. On the other hand, the regulatory challenge implicates the lack of harmonization in regulatory standards around the globe,[51] complexity of regulatory changes and discretion of health authorities to consent deviation from established guidelines. Therefore, a one-to-one comparison is often challenging and not necessarily justified.[52]

Conclusion

The acceptability of 3Rs concept is based on good manufacturing practices, good in-process quality control and validated procedures and processes. However, it is easier for new vaccines to adopt this concept. Significantdevelopments have been made in the improvement, maintenance, and upgradation of in-vitro potency assays likeELISA, Gel electrophoresis, Cell culture etc. which minimize the animal use and suffering.However, several difficulties are still experienced in theimplementation of 3Rs principles for vaccine potency assays. Thus, insistent exploration is obligatory by bothhealth industries and regulatory agencies for the implementation and validation of robust 3Rs approaches around the globe.

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None.

Conflicts of interest

Authors declare no conflict of interest.

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