Validation of standard quality control methods, the newly developed bacterial PCR (polymerase chain reaction) method and vaccine safety tests. Bovine, Vaccines.

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Research focus area:	Animal Products, Quality and Value-adding

Aims of the project

To validate a newly developed bacterial PCR method

To validate standard sterility test methods

To validate in vitro safety tests for foot-and-mouth disease virus

Executive summary

Bacterial PCR

It has been demonstrated that conventional polymerase chain reaction (PCR) assays present a practical solution for the early detection of bacterial contamination in pharmaceutical production processes. Combining PCR assays with DNA intercalating dyes reduced the occurrence of false positive results caused by the presence of non-viable bacterial cells. Propidium monoazide (PMA) binding dye, which intercalate with DNA of damaged cells rendering the DNA insoluble, were utilized to distinguish between viable and non-viable bacterial cells, using universal primers that amplify a ±1.5 kb fragment of 16S rDNA. Results showed that the PCR methods were not only more expedient than the standard sterility test, but also more sensitive since it consistently detected low levels of contamination which could not be detected using the standard methods. A comparative sensitivity analysis performed using mixed cultures revealed the ability of PMA, used in combination with conventional PCR, to detect the presence of viable cells. The results of the PMA-PCR assay correlated with those

observed for the untreated control. It is clear that a higher level of sensitivity can be achieved using PMA combined with conventional PCR.

Growth promotion tests of microorganisms

Each batch of prepared bacterial culture medium must be tested for growth promotion using suitable microorganisms as per European pharmacopeia (EP, 2005). Portions of TG (Thioglycollate) medium, TSB (Tryptone Soya Broth), NB (Nutrient Broth), and (BTA) Blood agar plates were inoculated with 100 colony forming units (CFU) per milliliter (ml) of four different species of microorganisms (*E. coli, S. Aureus, S, typhi and Klebsiella)*. After incubation, clearly visible growth of all four species of microorganisms was observed indicating that the bacterial culture media are suitable for use.

Sterility Test Validation

It is important to determine if the material used for production of vaccines (antigens, cell seeds, medium etc.) that will be sterility tested, contains elements that will interfere with the growth of microorganisms within the bacterial culture media used for the test. After transferring the contents (1 ml) of the materials used in production to be tested to the bacterial culture medium, an inoculum of a small number of viable microorganisms (100 CFU/ml) was added to the various samples tested. Each sample was tested separately against the four different species of microorganisms. The growth promotion test was used as a positive control, and bacterial culture medium, with no inoculum of test product or microorganisms, as a negative control. After incubation, clearly visible growth of all four different microorganisms was observed in all samples, except ATV (Active Trypsin Versene), gentamycin and penicillin. Thus indicating that the product being tested possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without any modification. ATV contains sodium hydroxide which has antimicrobial activity and gentamycin and penicillin are antibiotics that show antimicrobial activity against the microorganisms tested, indicating that the test is not suitable for evaluation of these products.

Validation of Safety Tests

According to the European Pharmacopeia (2005), a proportion of each batch of bulk inactivated antigen representing at least 200 doses must be tested for freedom from infectious virus by inoculation into sensitive cell cultures. For validation of the safety test, different cell lines were used, which includes BHK (baby hamster kidney) and $IBRS_2$ (porcine kidney). The

antigen sample to be tested was spiked with a FMDV vaccine strain with known titer and the cells were then observed for CPE (cytopathogenic effect). In general, all controls with no seed virus were negative and all spiked samples showed CPE indicating that the test is valid.

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