88

Growth, re-isolation and titration of *M. gallisepticum* ts-11 and *M. synoviae* MSH vaccine strains – the need for an appropriately validated media.

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Experience with ts-11 and also MSH in laboratories (using media made in house or purchased) around the world suggests that these strains are unique requiring quality control of media to make sure that these strains can grow optimally in media rather than the media being quality controlled on other strains. The best way to do this is to have reference preparations of ts-11 and MSH to control media. The easiest way to do this is to get a large number of vaccine vials of the same serial stored at -70C. If a media cannot get the reference preparation to the expected titre then titration of these vaccines strains with that media is invalid. This effect of media can vary from batch to batch and has been as large as a 6 $\log_{10}$ depression of growth. There is some suggestion that this effect is labile (due to heat and/or time since preparation) and perhaps from the yeast extract. This media quality problem has caused some problems. Government laboratories testing potency can get media from our manufacturing facility to titrate vaccine or develop their own media. Re-isolation studies have been severely biased by media quality making conclusions about strain displacement unreliable. Conversely it is probably not a problem in antibiotic sensitivity testing and perhaps the vaccines strains can be adapted to other media formulations but whether this would also change the properties under investigation is not known. Technically, the gold standard for titration is CFU- this allows an error estimate to be made from the replicates. CCU calculations have been traditionally used in mycoplasma quantification of vaccines but they have an intrinsic problem with variable experimental error because these are technically easier and less susceptible to contamination during the later period of incubation. Automation of titration with dilution being done on plates is incompatible with an assumption in calculation of MPN (Poisson distribution) – that the samples are taken independent of each other. The dilution series needs to be done off the plate. At least with MPN there are usually 8 to 12 replicates per dilution. Some people have also used Spearman Karber calculations but with no replicates. Only one possible significand for titre is possible if it is done without replicates. In CCU determinations how do we read titration end points with only a partial colour change occurs? Especially in laboratory temperature sensitivity tests. (These need to be done in incubators with temperature control in the non-permissive incubator being ±0.1C). Also descriptions of media preparations often are not clear that glucose should not be autoclaved with phosphates.