

London, 18 September 2007
Doc. Ref. EMEA/CVMP/VICH/1052/2004

VICH Topic GL41

at Step 7

**GUIDELINE ON TARGET ANIMAL SAFETY: EXAMINATION OF LIVE VETERINARY
VACCINES IN TARGET ANIMALS FOR ABSENCE OF REVERSION TO VIRULENCE**

ADOPTION BY CVMP FOR RELEASE FOR CONSULTATION	10 November 2004
TRANSMISSION TO INTERESTED PARTIES	15 November 2004
END OF CONSULTATION	10 May 2005
SIGN-OFF AT STEP 6 BY STEERING COMMITTEE	August 2007
ADOPTION BY CVMP	13 September 2007
DATE FOR COMING INTO EFFECT	1 July 2008

VICH GL 41 (TARGET ANIMAL SAFETY) – REVERSION TO VIRULENCE

July 2007

For implementation at Step 7

TARGET ANIMAL SAFETY: EXAMINATION OF LIVE VETERINARY VACCINES IN TARGET ANIMALS FOR ABSENCE OF REVERSION TO VIRULENCE

Recommended for Adoption
at Step 7 of the VICH Process
in July 2007 by the VICH SC
for implementation in July 2008

This Guideline has been developed by the appropriate VICH Expert Working Group and is subject to consultation by the parties, in accordance with the VICH Process. At Step 7 of the Process the final draft will be recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

Table of Contents

1. INTRODUCTION

1.1. Objective

1.2. Scope and general principle

2. STUDY DESIGN

3. GLOSSARY

1. INTRODUCTION

The absence of reversion to or increase in virulence test is generally an essential requirement for the registration or licensure of live vaccines in the EU, Japan and the USA. International harmonization of this test will minimize the need to perform separate studies for regulatory authorities of different countries. Appropriate international standard methods will reduce research and development costs by avoiding, whenever possible, duplication of tests. Animal welfare will benefit because fewer animals will be needed by eliminating repetition of similar tests in each region.

This guideline has been developed under the principle of VICH and will provide a unified standard for government regulatory bodies to facilitate the mutual acceptance of reversion to virulence data by the relevant authorities. The use of this VICH guideline to support registration of a product for local distribution only is strongly encouraged but is up to the discretion of the local regulatory authority. Furthermore, it is not always necessary to follow this guideline when there are scientifically justifiable reasons for using alternative approaches.

1.1. Objective

This guideline establishes agreed criteria and requirements for the conduct of studies that examine the potential for reversion to or increase in virulence of live veterinary vaccines in target animals.

1.2. Scope and General Principle

This guideline is intended to cover live vaccines. Live vaccines ⁽¹⁾ are those that may be capable of replication in the target animal, stimulate a useful immune response, and generally cannot be completely characterized by chemical and physical tests alone. The guideline covers the following species: bovine, ovine, caprine, feline, canine, porcine, equine, poultry (chickens and turkeys). This guideline will not provide information for the design of tests in other species including aquatic animals. For other species, tests should be designed following local guidance. Guidance on laboratory tests to determine adequate attenuation of the vaccine strain is not within the scope of this guideline.

(1) In case of vector vaccines this only covers vector vaccine seeds that replicate in the target species.

2. STUDY DESIGN

This study is carried out using the master seed. If the quantity of the master seed sufficient for testing is not available, the lowest passage seed used for production that is available in sufficient quantity should be examined. Use of another passage option must be justified. Generally, serial passages should be made in target animals through five groups of animals, unless there is justification to make more passages or the organism disappears from the test animals sooner. The time interval between inoculation of the animal and harvest for each passage must be justified based upon the characteristics of the test organism. If recovery is successful, passages should continue through five groups of animals. Appropriate methods, preferably *in vitro* propagation, should be used to confirm the presence and to determine the number of the test organisms at each passage. *In vitro* propagation may not be used to expand the passage inoculum.

Where a reasonable explanation for the sudden loss of the organism exists, e.g. experimental error, the previous passage may be repeated. When the organism is not recovered from any intermediate *in vivo* passage, a reasonable attempt should be made to repeat the test in 10 animals (90% probability of isolating the organism at 20% probability of recovery – see Appendix) using *in vivo* passaged material from the last passage in which the organism was recovered. If the target organism is recovered from one or more animals in the repeat test, the passages should continue using the material recovered in the repeat test as the inoculum for the next passage. The repeat test will be counted as a passage. If the target organism is not recovered, the experiment is considered to be completed with the conclusion that the target organism does not show an increase in or reversion to virulence.

Generally, for each target species, the most sensitive class, age, sex and serological status of animals should be used. In cases where alternative approaches are used, alternatives should be justified. Generally, a minimum of two animals is used for the first four groups and a minimum of eight animals is used for the fifth group.

Housing and husbandry should be adequate for the purpose of the study and conform to local animal welfare regulations. Animals should be appropriately acclimatized to the study conditions. Appropriate prophylactic treatment should be completed before the initiation of the study. Reduction or elimination of suffering during the study is essential. Euthanasia and necropsy of moribund animals is recommended.

The initial administration and subsequent passages shall be carried out using a recommended route of administration or natural route of infection that is the most likely to lead to reversion to or increase in virulence and result in recovery of the organism following replication in the animal. The route used must be justified.

The initial inoculum should contain the maximum release titer expected in the recommended dose or, in the cases where the maximum release titer to be licensed is not specified, then a justifiable multiple of the minimum release titer can be used. Passage inocula should be collected and prepared from the most likely source of spread of the organism, unless there is scientific justification to use another material.

General clinical observations should be made during the study. Animals in the fifth group should be observed for 21 days unless otherwise justified. These observations should include all relevant parameters typical for the disease which could indicate reversion to or increase in virulence. If signs consistent with the target disease are observed, then causality needs to be investigated. No evidence of an increase in virulence, indicative of reversion, should be seen with passage.

If the fifth group of animals shows no evidence of an increase in virulence indicative of reversion during the observation period, further testing is not required. Otherwise, materials used for the first passage and the final passage should be used in a separate experiment using at least 8 animals per group to directly compare the clinical signs and other relevant parameters. This study should be done by the route of administration that was used for previous passages. An alternative route of administration may be used if scientifically justified.

When attenuation of a test organism is known to be the result of a well characterized specific marker or genetic change, additional tests using suitable molecular biological methods for comparison of the initial seed organism and the organism recovered from the final passage should be performed, thus confirming the genetic stability of the attenuation marker in the vaccine strain.

If available data or assessment indicate a substantial risk that the test organism may revert to or increase in virulence, additional studies may be required to provide further information on the organism.

Except in exceptional and justified cases, if the completed studies show that the test organism does revert to or increase in virulence after passage in the target animal, the test organism will be deemed to be unsuitable for use as a live vaccine.

3. GLOSSARY

Class. Subset of target animal species which is characterized by factors such as reproductive status and/or use (dairy vs. beef, broiler vs. layer)

Master seed. A collection of aliquots of a micro-organism suspension for use in the preparation of the product, obtained from single culture, distributed from a single bulk into containers and processed together in a single operation in such a manner as to ensure uniformity and stability.

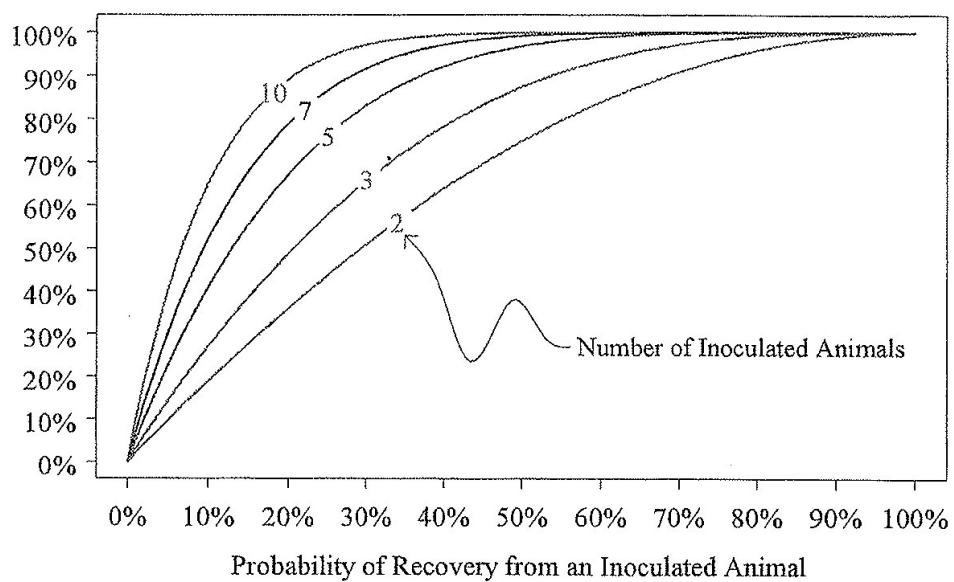
Maximum release titer. The expected highest number of viable organisms allowed per dose in vaccines at the time of release, verified by safety studies. In regions where a maximum release potency is not established, a justifiable multiple of the release antigen content is applied.

Minimum release titer. The expected lowest number of viable organisms required per dose in vaccines at the time of release, verified by efficacy and stability data.

Passage. Transfer of organisms through a group of inoculated animals, either from the beginning seed material or from a previous passage in animals.

APPENDIX

Probability of Recovery from At Least One Animal of a Group of Inoculated Animals



APPENDIX

The table gives the probability that the organism will be recovered from at least one animal of a group of inoculated animals. The probability of recovery from a single animal is in the left margin. The number of inoculated animals is in the top margin. The corresponding entry shows the probability that the organism will be recovered from at least one animal in the group.

Probability of Recovery from an Inoculated Animal	Number of Inoculated Animals				
	2	3	5	7	10
0.975	0.999	>0.999	>0.999	>0.999	>0.999
0.950	0.997	>0.999	>0.999	>0.999	>0.999
0.925	0.994	>0.999	>0.999	>0.999	>0.999
0.900	0.990	0.999	>0.999	>0.999	>0.999
0.875	0.984	0.998	>0.999	>0.999	>0.999
0.850	0.978	0.997	>0.999	>0.999	>0.999
0.825	0.969	0.995	>0.999	>0.999	>0.999
0.800	0.960	0.992	>0.999	>0.999	>0.999
0.775	0.949	0.989	0.999	>0.999	>0.999
0.750	0.938	0.984	0.999	>0.999	>0.999
0.725	0.924	0.979	0.998	>0.999	>0.999
0.700	0.910	0.973	0.998	>0.999	>0.999
0.675	0.894	0.966	0.996	>0.999	>0.999
0.650	0.878	0.957	0.995	0.999	>0.999
0.625	0.859	0.947	0.993	0.999	>0.999
0.600	0.840	0.936	0.990	0.998	>0.999
0.575	0.819	0.923	0.986	0.997	>0.999
0.550	0.798	0.909	0.982	0.996	>0.999
0.525	0.774	0.893	0.976	0.995	0.999
0.500	0.750	0.875	0.969	0.992	0.999
0.475	0.724	0.855	0.960	0.989	0.998
0.450	0.698	0.834	0.950	0.985	0.997
0.425	0.669	0.810	0.937	0.979	0.996
0.400	0.640	0.784	0.922	0.972	0.994
0.375	0.609	0.756	0.905	0.963	0.991
0.350	0.577	0.725	0.884	0.951	0.987
0.325	0.544	0.692	0.860	0.936	0.980
0.300	0.510	0.657	0.832	0.918	0.972
0.275	0.474	0.619	0.800	0.895	0.960
0.250	0.437	0.578	0.763	0.867	0.944
0.225	0.399	0.535	0.720	0.832	0.922
0.200	0.360	0.488	0.672	0.790	0.893
0.175	0.319	0.438	0.618	0.740	0.854
0.150	0.277	0.386	0.556	0.679	0.803
0.125	0.234	0.330	0.487	0.607	0.737
0.100	0.190	0.271	0.410	0.522	0.651
0.075	0.144	0.209	0.323	0.421	0.541
0.050	0.097	0.143	0.226	0.302	0.401
0.025	0.049	0.073	0.119	0.162	0.224