NOTIFICATION OF EXCLUSION OF ATTENUATED STRAINS

The *Public Health Security and Bioterrorism Preparedness and Response Act of 2002* requires the United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) to establish regulations regarding the possession, use, and transfer of select biological agents and toxins. In accordance with the Act, HHS and USDA published new regulations in the Federal Register on December 13, 2002 (67 FR 76886-76905 and 67 FR 76908-76938, respectively). The HHS regulations are set out in 42 CFR Part 73 and the USDA regulations are set out in 7 CFR Part 331 and 9 CFR Part 121.

The regulations in 42 CFR Part 73 and 9 CFR Part 121 establish a procedure by which an attenuated strain of a select biological agent or toxin that does not pose a severe threat to public health and safety, animal health, or animal products may be excluded from the list of select biological agents and toxins.

HHS has received requests for exclusions for *Yersinia pestis* strains, *Bacillus anthracis s*trains, *Francisella tularensis* subspecies *novicida* and *Francisella tularensis* subspecies *holartica* LVS.

USDA has received requests for exclusions for *Bacillus anthracis,* Sterne strain, *Francisella tularensis* subspecies *holartica* LVS, Rift Valley fever virus, MP-12 vaccine strain, Venezuelan equine encephalitis virus, TC-83 strain, Japanese encephalitis virus, SA14-14-2 strain, and recombinants of highly pathogenic avian influenza virus.

Based upon consultations with subject matter experts and a review of relevant published studies and information provided by the entities requesting the exclusions, HHS and USDA have determined that the following attenuated strains are not subject to the requirements of 42 CFR Part 73 and 9 CFR Part 121 if used in basic or applied research, as positive controls, for diagnostic assay development, proficiency testing, or for the development of vaccines and therapeutics.

However, an individual or entity that possesses, uses, or transfers an excluded attenuated strain will be subject to the regulations if there is any reintroduction of factor(s) associated with virulence or other manipulations that modify the attenuation such that virulence is restored or enhanced.

Attenuated strains of Overlap biological select agents and toxins excluded:

*Bacillus anthracis s*trains devoid of both plasmids pX01 and pX02. (effective 2-27-2003)

Bacillus anthracis strains devoid of the plasmid pX02 (e.g., *Bacillus anthracis* Sterne, $pX01^{+}pX02^{-}$). (effective 2-27-2003)

Brucella abortus strain RB51 (vaccine strain). (effective 5 -7-2003)

Brucella abortus Strain 19 (effective 6 -12-2003)

Coxiella burnetii Phase II, Nine Mile Strain, plaque purified clone 4. (effective 10-15-2003)

Francisella tularensis subspecies *novicida* (also referred to as *Francisella novicida*) strain, Utah 112 (ATCC 15482). (effective 2-27-2003)

Francisella tularensis subspecies *holartica* LVS (live vaccine strain; includes NDBR 101 lots, TSI-GSD lots, and ATCC 29684). (effective 2-27-2003)

Francisella tularensis ATCC 6223 (also known as strain B38). (effective 4-14-2003)

Rift Valley fever virus, MP-12 vaccine strain (effective 3-16-04)

Venezuelan equine encephalitis virus, TC-83 strain. (effective 3-13-2003)

Venezuelan equine encephalitis (VEE) virus vaccine candidate strain V3526. (effective 5-5-2003)

Attenuated strains of USDA select biological agents and toxins excluded:

Highly pathogenic avian influenza (HPAI) virus, recombinant vaccine reference strains of the H5N1 and H5N3 subtypes (effective 5-7-2004)

Japanese encephalitis virus, SA14-14-2 strain. (effective 3-12-2003)

BACKGROUND

Avian Influenza virus

Several recombinant reference vaccine strains of highly pathogenic subtypes have been excluded based on results from *in-vitro* and *in-vivo* studies indicating that these strains were not pathogenic in avian species. The data requirements necessary for exclusion consideration under 9 CFR 121.3 (g) can be downloaded by clicking here. Specific reference vaccine strains have not been listed here for proprietary reasons.

***Bacillus anthracis*:**

(1) *Bacillus anthracis* strains that are devoid of both virulence plasmids, pX01 and pX02 are excluded based on published studies evaluating the attenuation of strains containing different combinations of the two plasmids.

(2) *Bacillus anthracis* strains lacking the virulence plasmid pX02 (e.g., Sterne $pX01^+$ and $pX02^-$) are excluded based on information indicating that these strains were 10^5 - to 10^7-fold less virulent than isogenic strains with both plasmids. These strains have been used to vaccinate both humans and animals and do not pose a severe threat to the public health and safety.

References:

Human live anthrax vaccine in the former USSR, E. N. Shlyakhov and E. Rubenstein, *Vaccine*, Vol 12, No. 8, 1994, pages 727-730.

Avirulent Anthrax Vaccine, Max Sterne, *Onderstepoort Journal*, Vol. 21, No. 1, 1946, pages 41-43.

Anthrax: The Disease in Relation to Vaccines, P. Hambleton et al., *Vaccine*, Vol. 2, June 1984, pages 125-132.

***Brucella abortus*:**

(1) *Brucella abortus* strain RB51 was conditionally licensed as a vaccine by USDA in 1996 and granted a full license in March 2003. It is used as part of the cooperative State-Federal Brucellosis Eradication Program.[1] Brucella abortus strain RB51 is a genetically stable, rough morphology mutant of field strain Brucella. It lacks the polysaccharide O-side chains on the surface of the bacteria. Strain RB51 is less virulent than the Brucella abortus Strain 19 vaccine and field strain[2] Brucella abortus. The RB51 strain does not pose a significant threat to human or animal health.

References:

1. Brucellosis http://www.aphis.usda.gov/vs/nahps/brucellosis/

2. Schurig GG, Roop RM II, Bagchi T, Boyle S, Buhrman D, Sriranganathan N. Biological properties of RB51: a stable rough strain of Brucella abortus. Vet Microbiol 1991, 28:171-88.

3. Stauffer B, Reppert J, Van Metre D, Fingland R, Kennedy G, Hansen G, Pezzino G, Olsen S, Ewalt D. Human Exposure to Brucella abortus Strain RB51 – Kansas, 1997. MMWR 1998 47(09):172-175.

(2) The *Brucella abortus* Strain 19 live vaccine, used in the U.S. Department of Agriculture Brucellosis Eradication Program from 1941 to 1996, is effective in the control of clinical brucellosis in cattle.[1] For over a decade, *B. abortus* Strain 19 was also used to immunize more than 8 million people in the USSR.2 While there have been occasional reports of human brucellosis caused by *B. abortus* Strain 19 as a result of accidental aerosolization or needle sticks,[3, 4] this strain does not pose a severe threat to human or animal health.

References:

1. Proceedings of the United States Animal Health Association 93:640-655.
2. Joint FAO/WHO Expert Committee on Brucellosis, 1986. No. 740, p. 34-40.
3. Young, E., 1983. Human Brucellosis in Reviews of Infectious Diseases, Vol. No 5.
4. Pivnick, H, et al. 1966. Infection of Veterinarians in Ontario by *Brucella abortus* St 19.

Coxiella burnetii

(1) *Coxiella burnetii* Phase II, Nine Mile Strain, plaque purified clone 4. LPS is the only confirmed virulence factor of *C. burnetii*. Organisms isolated from natural infections or laboratories are in phase I and have a smooth-type LPS. Repeated passage of phase I organisms through embryonated eggs or cultured cells resulted in the conversion to phase II and a change in the LPS to a rough-type. Injection of such laboratory-derived phase II variants into guinea pigs resulted in infection and reversion to phase I. However, plaque-purified (cloned) isolates of the Nine Mile Strain phase II organisms do not undergo phase reversion and are avirulent as inoculation of susceptible animals with phase II cells does not result in infection nor can viable phase II or phase I organisms be recovered from the spleens of these animals. The Nine Mile Strain plaque purified phase II is stable and does not revert to phase I; restriction fragment length polymorphisms detected after *Hae*III digestion of chromosomal DNA and DNA-DNA hybridization, suggests that the Nine Mile Strain plaque purified phase II variant has undergone a deletion. Based upon consultations with subject matter experts and a review of relevant published studies, HHS and USDA have determined that *Coxiella burnetii*, Phase II, Nine Mile Strain, plaque purified clone 4 does not pose a significant threat to human or animal health.

References:

O'Rourke, A.T., M.. Peacock, J.E. Samuel, M.E. Frazier, D.O. Natvig, L.P. Mallavia, and O. Baca. 1985. Genomic analysis of phase I and II *Coxiella burnetii* with restriction endonucleases. J. Gen. Microbiol. 131:1543-1546.

Vodkin, M.H., J.C. Williams, and E.H. Stephenson. 1986. Genetic heterogeneity among isolates of *Coxiella burnetii.* J. Gen. Microbiol. 132:455-463.

Moos, A. and T. Hackstadt. 1987. Comparative virulence of intra- and interstrain lipopolysaccharide variants of *Coxiella burnetii* in the guinea pig model. Infect. Immun. 55:1144-1150.

Francisella tularensis

(1) The type strain Utah 112 of *Francisella tularensis* subspecies *novicida* (also referred to as *Francisella novicida*) is excluded. The exclusion is only for the type strain, Utah 112. This strain was originally isolated from a water sample taken from Ogden Bay, Utah in 1951. It is experimentally pathogenic for mice, guinea pigs and hamsters, producing lesions similar to those of tularemia; rabbits, white rats and pigeons are resistant. The Utah 112 strain is not known to infect man and thus, is not of public health concern.

(2) *Francisella tularensis* subspecies *holartica* LVS (live vaccine strain) is excluded. This strain has been used to vaccinate millions of persons in the Former Soviet Union and thousands of U.S. military personnel and laboratory workers without major problems.

(3) *Francisella tularensis* biovar *tularensis* strain ATCC 6223. This strain has fastidious growth requirements and grows poorly in the laboratory. Mice are used as a model to study the pathogenesis of tularemia. The LD50 of virulent strains of *F.* tularensis biovar *tularensis* for mice infected via the subcutaneous route is <10 CFU. However, Mice infected intraperitoneally with 105 CFU or intradermally with 107 CFU of strain ATCC 6223 were not killed. Thus, strain ATCC 6223 does not pose a threat to human or animal health.

Reference:

Tularemia, by J. Ellis et al, *Clinical Microbiological Reviews*, Vol 15, No. 4, Oct 2002, pp. 631-646.

Japanese encephalitis virus

(1) Japanese encephalitis virus, SA14-14-2 strain is excluded. This strain is the vaccine strain of choice in the People's Republic of China to protect against Japanese encephalitis. It is non-pathogenic in weanling mice and rhesus monkeys.

Reference:

Japanese encephalitis: a Chinese solution?, *The Lancet*, Vol. 347, June 1996, p. 1570.

Japanese encephalitis virus live-attenuated vaccine, Chinese strain SA14-14-2; adaptation to primary canine kidney cell cultures and preparation of a vaccine for human use, *Vaccine*, Vol. 6, Dec. 1988, pp. 513-518.

Rift Valley fever virus

(1) Rift Valley fever virus, MP-12 vaccine strain is excluded. Currently, under 42 CFR Part 73 MP-12 is excluded. The Rift Valley fever strain MP-12 is a live-attenuated vaccine, which was developed by serial mutagenesis. This strain does not pose a significant threat to human or animal health.

References:

1. Caplen, H., et. Al. 1985. Mutagen-directed Attenuation of Rift Valley Fever Virus as a Method for Vaccine Development. J. Gen. Virol. 66:2271-2277.

2. Morrill, J.C., et. al. 1991. Further evaluation of a mutagen-attenuated Rift Valley fever vaccine in sheep. Vaccine 9: 35-41.

3. Morrill, J.C., et. al. 1997. Safety of a mutagen-attenuated Rift Valley fever virus vaccine in fetal and neonatal bovids. AJVR 58: 1110-1114.

4. Morrill, J.C., et. al. 1997. Safety and efficacy of a mutagen-attenuated Rift Valley fever virus vaccine in cattle. AJVR 58: 1104-1109.

5. Morrill, J.C., et. al. 1987. Pathogenicity and immunogenicity of a mutagen-attenuated Rift Valley fever virus immunogen in pregnant ewes. AJVR 48: 1042-1047.

6. Rossi, C.A. and Turell, M.J. 1988. Characterization of Attenuated Strains of Rift Valley Fever Virus. J. Gen. Virology 69: 817-823.

7. Saluzzo, J.F. and Smith, J.F. 1990. Use of reassortant viruses to map attenuating and temperature-sensitive mutations of the Rift Valley fever virus MP-12 vaccine. Vaccine 8: 369-375.

Venezuelan equine encephalitis virus

(1) Venezuelan equine encephalitis virus, TC-83 strain is excluded. This strain is used in the production of USDA licensed equine biologics and is used as a human vaccine.

Reference:

Long-term duration of detectable neutralizing antibodies after administration of live-attenuated VEE vaccine and following booster vaccination with inactivated VEE vaccine. *Vaccine*, Vol. 14, No.4, 1996, pp. 337-343.

(2) Venezuelan Equine Encephalitis (VEE) strain V3526 is an attenuated strain of VEE, which was constructed by site-directed mutagenesis. V3526 contains two mutations relative to the virulent parental clone [(1)]. One of these mutations is a deletion, which renders the virus non-viable; the other mutation restores viability without restoring the pathogenic properties of the parental virus. The stability of the deletion mutation in V3526 fundamentally and significantly decreases the hazard associated with this strain, and makes it unlikely that it can revert to wild type. This strain is considerably less virulent than the excluded vaccine strain TC-83. This strain does not pose a significant threat to human or animal health.

References:

1. Davis, N.L., et al. 1995. Attenuated mutants of Venezuelan equine encephalitis virus containing lethal mutations in the PE2 cleavage signal combined with a second site suppressor mutation in E1. Virology 212:102-110.