参考資料 2－3

米国において規制対象から除外されている病原体等

※ 感染症法案での規制対象のうち、米国 HHS/USDA において、規制対象の特別物質・毒素(Select Agent and Toxin)から除外されている病原体等は次のとおり。

＜HHS 除外の弱毒株＞

- アレナウイルス属フニンウイルス ワクチン株 Candid 1
- エルシニア属ペスティス(ペスト菌) EV76 株、Lcr プラスミド欠損株

＜HHS/USDA 除外の弱毒株＞

- バシラス属アントラシス(炭疽菌)pX01 及びpX02 両プラスミド欠損株、pX02 プラスミド欠損株
- ブルセラ属菌アボルタス 19 株、RB51 株
- コクシエラ属バーネッティイ Phase Ⅱ Nine Mile 株(plaque purified clone 4)
- フランシセラ属ツラレンシス(野兎病菌)亜種ホルアークティカ LVS 株(生ワクチン株)
- フランシセラ属ツラレンシス(野兎病菌)亜種ツラレンシス ATCC6223(B38 株)
- フィレボウイルス属リフトバレーフィーバーウイルス(リフトバレー熱ウイルス)MP-12 ワクチン株
- アルファウイルス属ヴェネズエラエクインエンセファリティスウイルス(ベネズエラ馬脳炎ウイルス)ワクチン株 V3526、ワクチン株 TC-83

＜許容される毒素量＞

主任研究者、医師、獣医師等の管轄する毒素で、総量が、いかなる時点でも以下の規定量を超えないもの。

- ボツリヌス毒素(神経毒素) 0.5mg、志賀毒素 100mg

米国における特別物質の除外等について

※ 米国 National Select Agent Registry よりHHS特別物質、重複特別物質部分を抜粋
(http://www.selectagents.gov/exclusions.htm)

Select Agent Exclusions

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* strains, *Francisella tularensis* subspecies *novicida* and *Francisella tularensis* subspecies *holartica* LVS.

USDA has received requests for exclusions for *Bacillus anthracis* Sterne strain and *Francisella tularensis* subspecies *holartica* LVS.

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, 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 HHS select agents and toxins excluded:

- ***Coccidioides posadasii* Δchs5 strain.** (effective 10-14-2003)

The data demonstrates that deletion of the single copy of the class V chitin synthase using a gene replacement strategy results in a stable avirulent phenotype that lacks the ability to form infectious arthroconidia. This mutant is unable to form spherules in vivo as exemplified by its inability to survive or kill mice following intraperitoneal inoculation. Based upon consultations with subject matter experts and information

provided by the requestor, HHS has determined that *Coccidioides posadasii* Δchs5 mutant strain does not pose a severe threat to public health and safety.

- **Conotoxins** specifically excluded are: the class of sodium channel antagonist μ-conotoxins, including GIIIA; the class of calcium channel antagonist ω-conotoxins, including GVIA, GVII, MVIIA, MVIIC, and their analogs or synthetic derivatives; the class of NMDA-antagonist conantokins, including con-G, con-R, con-T and their analogs or synthetic derivatives; and the putative neurotensin agonist, contulakin-G and its synthetic derivatives. (effective 4-29-2003)

  The term "conotoxin" is used broadly to comprise a very large number of polypeptides isolated from the venom of fish-hunting marine snails of the Conus genus of gastropod mollusks; many of these molecules are neurologically active in mammals [1-3]. Based upon available experimental evidence, however, the following conotoxins (i.e. conopeptides) do not possess sufficient acute toxicity to pose a significant public health threat, and many are employed as useful research tools or potential human therapeutics. Select Agent conotoxins excluded are: the class of sodium channel antagonist μ-conotoxins, including GIIIA [3]; the class of calcium channel antagonist ω-conotoxins, including GVIA, GVII, MVIIA, MVIIC, and their analogs or synthetic derivatives [3,4]; the class of NMDA-antagonist conantokins, including con-G, con-R, con-T and their analogs or synthetic derivatives [5]; and the putative neurotensin agonist, contulakin-G and its synthetic derivatives [6].

References:

1. Olivera, B.M., Gray, W.R., Zeikus, R., McIntosh, J.M., Varga, J., Rivier, J., de Santos, V. and Cruz, L.J. (1985). Peptide neurotoxins from fish-hunting cone snails. Science 230:1338-1343.
2. Olivera, B.M., Rivier, J., Scott, J.K., Hillyard, D.R. and Cruz, L.J. (1991). Conotoxins. J. Biol. Chem. 266:22067-22070.
3. Olivera, B.M. and Cruz, L.J. (2001). Conotoxins, in retrospect. Toxicon 39:7-14.
4. Yoshikami, D., Bagabaldo, Z. and Olivera, B.M. (1989). The inhibitory effects of omega-conotoxins on Ca channels and synapses. Ann. N.Y. Acad. Sci. 560:230-248.
5. Prorok, M. and Castellino, F.J. (2001). Structure-function relationships of the NMDA receptor antagonist conantokin peptides. Curr. Drug Targets 2:313-322.
6. Craig, A.G., Norberg, T., Griffin, D., Hoeger, C., Akhtar, M., Schmidt, K., Low, W., Dykert, J., Richelson, E., Navarro, V., Mazella, J., Watkins, M., Hillyard, D., Imperial, J., Cruz, L.J., and Olivera, B.M. (1999). Contulakin-G, an O-glycosylated invertebrate neurotensin. J. Biol. Chem. 274:13752-13759.

- **Junin virus** vaccine strain Candid 1. (effective 2-7-2003)

- ***Yersinia pestis* strains** which are Pgm- due to a deletion of a 102-kb region of the chromosome termed the pgm locus (i.e., Δpgm). Examples are *Y. pestis* strain E.V. or various substrains such as EV 76. (effective 3-14-2003)

  Pgm- mutants of *Yersinia pestis* occur at a high frequency (ca 10-5) (1) and result in avirulence and Pgm- strains such as the EV 76 strain have been used for years as live human vaccines with no significant plague-associated problems. The mutation in question is due to the excision of about 102-kb of chromosomal DNA via reciprocal recombination between adjacent IS 100 elements (2). The lost DNA sequence encodes the ability to synthesize and utilize the siderophore yersiniabactin, which is necessary for growth in mammalian peripheral tissue, as well as the Hms+ locus, which is necessary for biofilm production in the flea vector (3). However, PCR and/or Southern blot analysis will be required to ensure that "Pgm-" derivatives have undergone this deletion rather than a mutation in the hemin storage genes (hms), which also causes loss of Congo red (CR) binding, which is the most common characteristic used to evaluate the pigmentation phenotype (4).

References:

1. Brubaker, R. R. 1970. Mutation rate to nonpigmentation in Pasteurella pestis. J. Bacteriol. 98:1404-1406.
2. Fetherston, J.D., P. Scheutze, and R.D. Perry. 1992. Loss of the pigmentation phenotype in *Yersinia pestis* is due to the spontaneous deletion of 102 kb of chromosomal DNA which is flanked by a repetitive element. Mol. Microbiol. 6:2693-2704.
3. Bearden, S.W., and R.D. Perry. 1999. The Yfe system of *Yersinia pestis* transports iron and manganese and is required for full virulence of plague. Mol. Microbiol. 32:403-414.
4. Une, T. and R.R. Brubaker. 1984. In vivo comparison of avirulent Vwa- and Pgm- or Pstr phenotypes of Yersiniae. Infect. Immun. 43:895-900.

- ***Yersinia pestis*** strains (e.g., Tjiwidej S and CDC A1122) devoid of the 75 kb low-calcium response (Lcr) virulence plasmid. (effective 2-27-2003)

  Strains of *Yersinia pestis* that lack the 75 kb low-calcium response (Lcr) virulence plasmid are excluded. Strains lacking the Lcr plasmid (Lcr-) are irreversibly attenuated due to the loss of a virulence plasmid. An Lcr- strain of *Yersinia pestis* (Tjiwidej S) has been extensively used as a live vaccine in humans in Java. Thus, these strains pose no significant threat to public health.

References:

1. Plague immunization. I. Past and present trends, K.F. Meyer et al., J. Infect. Dis. 1974; 129 (suppl.): S13-S18.

Attenuated strains of Overlap select agents and toxins excluded:

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

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 105- to 107-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:

3. Human live anthrax vaccine in the former USSR, E. N. Shlyakhov and E. Rubenstein, Vaccine, Vol 12, No. 8, 1994, pages 727-730.
4. Avirulent Anthrax Vaccine, Max Sterne, Onderstepoort Journal, Vol. 21, No. 1, 1946, pages 41-43.
5. Anthrax: The Disease in Relation to Vaccines, P. Hambleton et al., Vaccine, Vol. 2, June 1984, pages 125-132.

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

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 105- to 107-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:

3. Human live anthrax vaccine in the former USSR, E. N. Shlyakhov and E. Rubenstein, Vaccine, Vol 12, No. 8, 1994, pages 727-730.
4. Avirulent Anthrax Vaccine, Max Sterne, Onderstepoort Journal, Vol. 21, No. 1, 1946, pages 41-43.
5. Anthrax: The Disease in Relation to Vaccines, P. Hambleton et al., Vaccine, Vol. 2, June 1984, pages 125-132.

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

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. 5, No 5.
4. Pivnick, H, et al. 1966. Infection of Veterinarians in Ontario by *Brucella abortus* St 19.

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

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 strain2 *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.

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

LPS is the only confirmed virulence factor of *C. burnetii*. Organisms isolated from natural infections or laboratory 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 since 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 HaeIII 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:

1. 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.
2. Vodkin, M.H., J.C. Williams, and E.H. Stephenson. 1986. Genetic heterogeneity among isolates of *Coxiella burnetii*. J. Gen. Microbiol. 132:455-463.
3. 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*** subspecies *novicida* (also referred to as Francisella *novicida*) strain, Utah 112 (ATCC 15482). (effective 2-27-2003)

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 and similar strains have been used to vaccinate millions of people including thousands of U.S. military personnel and laboratory workers without major problems.

References:

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

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 (1). The LD50 of virulent strains of *F. tularensis* biovar tularensis for mice infected via the subcutaneous route is <10 CFU (1). 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:

4. Ellis, J., P.C.F. Oyston, M. Green, and R.W. Titball. 2002. Tularemia. Clin. Microbiol. Rev. 15:631-646.

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

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 and similar strains have been used to vaccinate millions of people including thousands of U.S. military personnel and laboratory workers without major problems.

References:

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

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 (1). The LD50 of virulent strains of *F. tularensis* biovar tularensis for mice infected via the subcutaneous route is <10 CFU (1). 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.

References:

4. Ellis, J., P.C.F. Oyston, M. Green, and R.W. Titball. 2002. Tularemia. Clin. Microbiol. Rev. 15:631-646.

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

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 and similar strains have been used to vaccinate millions of people including thousands of U.S. military personnel and laboratory workers without major problems.

References:

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

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 (1). The LD50 of virulent strains of *F. tularensis* biovar tularensis for mice infected via the subcutaneous route is <10 CFU (1). 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.

References:

4. Ellis, J., P.C.F. Oyston, M. Green, and R.W. Titball. 2002. Tularemia. Clin. Microbiol. Rev. 15:631-646.

- **Rift Valley Fever (RVF) virus** vaccine strain MP-12. (effective 2-7-2003)

- **Venezuelan Equine Encephalitis (VEE) virus** vaccine candidate strain V3526. (effective 5-5-2003)

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 TC83. 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.

- **Venezuelan Equine Encephalitis (VEE) virus** vaccine strain TC-83. (effective 2-7-2003)

※ 米国 National Select Agent Registry よりHHS/USDA 許容毒素量を抜粋
(http://www.selectagents.gov/toxinLimits.htm)

Permissible Toxin Amounts

The following toxins are not regulated if the amount under the control of a principal investigator does not exceed, at any time, the amounts indicated in the table below.

HHS / USDA Overlap Toxins [§73.3(d)(3)]	**Amount**
Botulinum neurotoxins	0.5 mg
Staphylococcal enterotoxins	5.0 mg
Clostridium perfringens epsilon toxin	100 mg
Shigatoxin	100 mg
T-2 toxin	1000 mg