

**Data Necessary to Consider Select Agent Exclusion Requests
Attenuated Strains of Highly Pathogenic Avian Influenza (HPAI) Viruses**

Parameter	Method	Outcome Specification
Source of all genes in construct; description of modification	Reference source material for viruses, plasmids, etc.	Description of gene composition of recombinant/attenuated strain
Complete nucleotide sequence analysis of the entire HA gene and analysis of the amino acid motif at the HA cleavage site	Standard laboratory methods	Confirmation of expected sequence for attenuated strain. Demonstration of HA cleavage site that is consistent with low pathogenic virus.
Pathogenicity testing in chickens	As described in the current Office International des Epizooties (OIE) Manual of Standards for Diagnostic Tests and Vaccines.	Confirmation of low-pathogenic phenotype in chickens.
Plaque characterization on chicken embryo fibroblast (CEF) cells without trypsin*	Test duplicate dilutions of strain in CEF cells with and without trypsin.	Demonstration of inability to form clearly defined plaques in the absence of trypsin.
Plaque characterization on CEF cells with trypsin*	Determine PFU/ml of representative product.	Demonstration of ability to form viral plaques in the presence of trypsin.

*With prior approval from USDA, MDCK cells may be substituted for CEF cells in this test.