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Department of  
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## CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 13-05

Animal and Plant  
Health Inspection  
Service

Veterinary Services

Center for Veterinary  
Biologics

1920 Dayton Avenue  
PO Box 844  
Ames, IA 50010

(515) 337- 6100

**TO:** Biologics Licensees, Permittees, and Applicants  
Directors, Center for Veterinary Biologics  
Veterinary Services Leadership Team

**FROM:** Richard E. Hill, Jr. /s/ *Richard E. Hill, Jr.*  
Director  
Center for Veterinary Biologics

**SUBJECT:** Use of Polymerase Chain Reaction (PCR) Assays to Measure Potency of  
Inactivated Protein-Based Biologicals

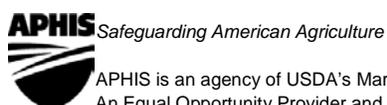
### I. PURPOSE

This Notice clarifies Center for Veterinary Biologics (CVB) policy regarding the use of PCR assays in the potency evaluation of inactivated protein-based biologicals.

### II. BACKGROUND

All serials of biological products must be tested for potency before their release for marketing. Most such serial release testing is done with assays that measure potency, although some utilize laboratory models based on efficacy surrogates or correlates, such as laboratory animal vaccination-challenge models or host species serology. Potency is a quantitative measure of a preparation's active ingredient. For vaccines, the active ingredient is the protective epitope. (This is a simplification that is useful for discussion, but we recognize that vaccine activity may be a more complex interplay between more than one epitope and other components such as adjuvants.) By providing a quantitative measure of the active component, an ideal potency assay has a direct relationship with efficacy.

Historically, most in vitro assays used to test the potency of inactivated protein-based biologics have been designed to quantify antigen. The target antigen is selected for its role in the protective immune response; preferably it is detected (measured) via an agent-neutralizing monoclonal antibody. This, combined with strict manufacturing controls, is intended to mitigate the risk associated with quantifying one representative antigen of a complex microbial agent in an in vitro platform.



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## **CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 13-05**

Page 2

Recently we have received several proposals to use PCR to test inactivated protein-based biologicals. PCR assays measure the presence of genes that *code for* proteinaceous antigens in vaccines. PCR assays do not provide assurance an antigen has actually been produced by a gene or that the antigen has retained its immunogenic form through inactivation and other downstream product processing. Many antigens, especially bacterial, are conditionally expressed, based on nutrient availability and/or environmental stressors. If unfavorable growth conditions down-regulate the expression of a critical antigen, this would not be detected at the nucleic acid level. Thus, PCR requires critical assumptions beyond those required for an antigen-quantification assay, and the CVB maintains that these assumptions represent excessive risk unless mitigating factors can be implemented.

One potential mitigating factor is to combine PCR with another assay(s) which, when considered together, provide superior assurance of serial potency than either assay alone. To date, the most frequent reason for considering PCR for potency testing is because a product contains multiple closely related microbial strains, and strain-specific reagents are not readily available to develop antigen-based assays that will not cross-react with other fractions in the product. In this case, the CVB will consider a cross-reacting protein-based assay to measure overall cross-reacting antigen content in conjunction with PCR to assess the probable relative input by each strain.

### **III. POLICY**

When PCR is proposed to test the potency of an inactivated protein-based biological, it shall be in the context of a multi-component potency assessment. One component of the assessment must characterize protein antigen in the completed product.

### **IV. IMPLEMENTATION/ APPLICABILITY**

This Notice simply clarifies existing CVB policy regarding the use of PCR in potency testing inactivated protein-based biologicals. No changes are being implemented.