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**United States Department of Agriculture**

Animal and Plant Health Inspection Service

Veterinary Services

Center for Veterinary Biologics

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# CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 12-04

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

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

**SUBJECT:** Appropriate Use of Controls for Chicken Anemia Virus Extraneous Agent Testing, Availability of a PCR-based Testing Protocol, and Availability of a New Testing Reagent

## I. PURPOSE

This Notice provides guidance on the use of controls in the testing for extraneous chicken anemia virus (CAV) that will provide valid test results for interpretation. It also provides information on the availability of a new polymerase chain reaction (PCR) based protocol, discussed in Veterinary Services Memorandum 800.89, for the detection of extraneous CAV. The new version of the protocol contains a new reagent for use as a positive control.

## II. BACKGROUND

Testing for extraneous CAV is recommended for firms in the case of Master Seed Viruses (MSV) prepared using poultry origin substrates. It is required for infectious bursal disease virus fractions propagated in chicken bursas and when outbreaks of CAV occur in CAV-negative specific pathogen free (SPF) flocks used in veterinary biologics preparation.

The Center for Veterinary Biologics (CVB) laboratory routinely tests for extraneous CAV using the current version of our protocol, entitled “Polymerase Chain Reaction Assay for Detection and Identity of Extraneous Chicken Anemia Virus (CAV)”, and designated VIRPRO0118.04. A copy is attached to this Notice. This protocol has been posted to our web site with other selected protocols; future versions of this protocol will be posted there or available upon request.

The current protocol makes use of internal and external positive controls to allow for the interpretation of negative test results. An internal control is added to the sample material being tested to ensure the sample material does not contain components that will inhibit or interfere with the amplification of CAV DNA. Inhibition of the PCR has been reported in the literature (Soine et al[11]) and observed in our laboratory (6/75 samples tested in Summer/Fall 2010). An external positive control is added to the PCR master mix to ensure amplification of the CAV DNA template by the reaction.

Several recent CAV outbreaks in CAV-negative SPF flocks have resulted in use of the CVB PCR protocol and the CVB's review of bench records provided by licensed firms. It was noted that specifics of the test method were not always on file with the CVB and positive controls were not always incorporated in the testing.

III. POLICY

When testing for extraneous CAV is conducted, the procedure used should be on file as a Special Outline and acceptable to the Animal and Plant Health Service (APHIS). A procedure acceptable to APHIS should include internal and external positive controls. In the case of a PCR-based test, the results of both the master mix (external control) and a spike for the material being tested (internal control) should be reported with test results relative to the presence or absence of CAV. If another test method is selected and acceptable to APHIS, the results of equivalent controls should be included in the Special Outline and reported.

An example of a procedure acceptable to APHIS has been developed by the CVB laboratory and the current version is documented in VIRPRO0118. In connection with this, a 419 base pair (bp) fragment of CAV, which can be used as an internal or external positive control for the procedure described in VIRPRO0118.04, was developed. Its usefulness for other protocols should be evaluated on a case by case basis. This reagent will be available in limited quantites as it is being incorporated into the PCR protocols selected by firms. After the supply is exhausted, firms will be expected to prepare their own lots. The protocol used to prepare this reagent, entitled "Production of Chicken Anemia Virus (CAV) DNA Positive Control" and designated VIRNPP0001.01, is also attached to this Notice. This will be available on our web site or by request, as well. Updated versions of the protocol will be placed on the web site. If comparable positive controls have been prepared by firms, they should feel free to use them.

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[1] Soine, C., Watson, S. K., Rybicki, E., Lucio, B., Nordgren, R. M., Parrish, C. R., and Schat, K. A, 1993. Avian Diseases 37: 467-476

Outlines indicating the use of CAV extranaeous agent testing should reference a Special Outline that provides the details for conducting the test. The Special Outline should specifiy the positive and negative controls used, and provide details on the nature of the controls. If a firm chooses to use the CVB protocol, the details of our protocol may be incorporated into a Special Outline maintained by the firm. If another protocol is used, data equivalent to our protocol should be submitted to support it.

IV. IMPLEMENTATION

The use of CAV positive controls (internal and external) for the testing for extraneous CAV is considered a requirement for a valid negative test result for the material being tested. Firms should review their procedures to ensure that positive controls are included in the protocols. This should be in place within 30 days of the publication of this Notice. Bench records for CAV testing may be audited by CVB during inspections or as the result of an outbreak and should clearly indicate the controls that are used. It should be possible to determine the nature of such controls from firm documentation.

Additionally, Outlines of Production should be revised and Special Outlines drafted to be consistent with these recommendations by the next annual review of the relative documents. Therefore, firms would be expected to be in compliance with this Notice within 18 months of publication of this Notice. Firms needing additional time to come into compliance with the filing of documents should contact their reviewer to discuss their particular situation.