

Reference

Haggard, D.L., Sherman, D.M.: Vaccine development in the prevention of bovine enteric colibacillosis. *The Compendium on Continuing Education*. Volume 6, number 6, June 1984, pp. S347-S353.



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VMRD, Inc.

Veterinary Medical Research and Development

P.O. Box 502
Pullman, WA 99163
USA

Orders: (800) 222-8673
Tech Support: (509) 334-5815
Fax: (509) 332-5356
Email: vtech@vmrd.com
Web: <http://www.vmrd.com>



VMRD, Inc.
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ESCHERICHIA COLI ANTIGEN TEST KIT

K99 Pilitest™

USDA Product Code 5032.00

General Information

Ten percent of beef and dairy calves are lost each year to scours. One of the most important infectious causes of scours in young calves is enteropathogenic *Escherichia coli* that carries K99⁺ pili. Fortunately, immunoprophylaxis has been shown to be effective to reduce the severity of scours caused by enteropathogenic *E. coli*. Nevertheless, because of the sporadic nature of outbreaks, the expense of vaccines and oral antibodies may not be justified without a specific etiologic diagnosis of K99⁺ *E. coli*.

Description

PILITEST™ is a sensitive latex agglutination test for the presence of K99⁺ *E. coli* in stools of diarrhetic calves. It may also be used for detection of K99⁺ *E. coli* grown on solid or liquid medium. The agglutination reaction takes less than 3 minutes and the entire test including sample preparation can be performed in 10 minutes or less.

Indications

PILITEST™ is recommended for use with stools from calves exhibiting signs of diarrhea or "white scours" within 5 days of birth. While it is possible to detect K99⁺ *E. coli* in stools of older calves, its contribution to diarrhea in this age group may not be significant.

Sensitivity of PILITEST™ is greater than 92% on stools of calves showing diarrhea for 24 hours or less and 89% on calves showing diarrhea for 48 hours or less. However, bacterial numbers fall in some calves after 48 hours. Therefore, when testing calves having diarrhea for more than 48 hours, it may be necessary to test several calves before concluding that K99⁺ *E. coli* are not responsible for the diarrhea observed.

Kit Contents

Plastic squeeze bottle "A" containing antibody coated latex beads, plastic squeeze bottle "B" containing suspended K99⁺ *E. coli* (positive control), plastic squeeze bottle "C" containing suspended K99⁻ *E. coli* (negative control), plastic squeeze bottle "D" containing control latex beads, 1 black slide with 4 rings, a supply of toothpicks and a photograph of reactions.

Specimen Requirements

Fresh stool sample from diarrhetic calf (approximately 0.5 ml) or *E. coli* on or in artificial medium.

Procedure

Sample Preparation. Handling of the sample prior to mixing with the latex can be done in a variety of ways—the major objective being to dilute the sample as little as possible while producing a homogenous sample of low viscosity that will not interfere with interpretation of the test. Viscous samples should be diluted with an equal volume of saline or water and mixed. The following are two suggestions to achieve separation of fecal fluid from solids:

- Syringe method—The sample may be drawn into a syringe after dilution (if necessary) with saline and carefully passed through a needle into the hub of which a small amount of cotton has been inserted. This will effectively filter out the solid material.
- Test tube method—Centrifuge the mixture at approximately 1000 times gravity (most serology centrifuges are adequate) for 5 minutes. The tube may also be left standing for about an hour if a centrifuge is not available. Collect the supernatant with a pipette or syringe.
- For detection of K99⁺ *E. coli* grown in/on artificial medium: Remove *E. coli* colony from agar with a loop and mix with 2 drops of saline or in liquid medium use 1 or 2 drops of log phase culture.

Once a homogenous sample has been obtained...

- 1—Shake all four squeeze bottles. Add one drop of "A" (antibody coated latex beads) to the first three rings and a drop of "D" (control latex beads) to the fourth ring on the black slide. Then, add a drop of the sample to the first and fourth rings, a drop of "B" (positive control antigen) to the second ring, and a drop of "C" (negative control antigen) to the third ring.
- 2—Mix the two drops in each ring with separate toothpicks for each ring.
- 3—Gently and continuously rock the slide for 2 minutes and observe for agglutination. Then allow the slide to remain still for an additional minute and observe again.

Interpretation

- Agglutination in ring one (sample) indicates infection with K99⁺ *E. coli* provided no agglutination occurs in rings three (negative control) and four (latex bead control).
- If no agglutination is observed in ring one (sample) within 3 minutes, it is considered negative provided agglutination occurs in ring two (positive control).
- Wash the black slide with water and wipe dry between tests. The black slide should be used in good light.
- Refer to the photograph below for examples of weak (Ring 1), strong (Ring 2) and negative reactions (Rings 3 and 4).
- If you have questions, please call our Technical Services Department at (509) 334-5815. We would be happy to help you.

