# Instructions For Use of the Bovine Virus Diarrhea Antigen Test Kit

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## Name and Use of Kit

The CST-QuickTest™ test kit is a sandwich ELISA that detects Bovine Virus Diarrhea Virus (BVDV) antigen in bovine ear notch samples.

## BVDV Information

Bovine Virus Diarrhea Virus causes economically important diseases worldwide. While BVDV infections are present in a variety of clinical diseases of varying severity, most infections result in subclinical disease.[1,2] Persistently Infected (PI) cattle are considered to be the principle reservoir of BVDV infections. PI calves develop in utero when the dam becomes viremic with BVDV between approximately 42 to 125 days of gestation. Infected fetuses that survive until birth are born immunotolerant to the specific exposing viral strain and are lifelong shedders of the virus, exposing all cattle that come into contact with the PI calf. PI cattle are considered to be the primary source of BVDV transmission and can result in significant economic losses in cattle populations.[3,4] Identification and removal of PI animals is a valuable tool in herd health and cattle feeding management.

## Principles of the Test

Paired pestivirus NS23 (p80/125) monoclonal antibodies are used to detect NS23 antigen of BVDV. One monoclonal coated to a microwell plate is used to capture the antigen. While the other monoclonal, conjugated with HRP allows for a colormetric reaction when mixed with a substrate if antigen is present.

## Reagents, References and Equipment Included

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| A) 6 Microwell test plates | 6x96 microwell plates |
| 1) 1 hinged vial Positive Control* | 3 mLs |
| 2) 1 hinged vial Negative Control*+ | 3 mLs |
| 3) 1 bottle Working Conjugate+ | 34 mLs |
| 4) 1 bottle TMB Substrate | 83 mLs |
| 5) 1 bottle ProClin 300 | 900 µLs |
| 6) 2 hinged vials Extraction Buffer Dry Mix | 5.89 gms |

* Contains ProClin 300 as preservative + Contains StabilZyme as preservative

## Storage

Diagnostic kit should be stores at 2-8°C. Mixed Extraction Buffer can be stored at 18-24°C for up to 12 hours; 2-8°C for up to 14 days; or frozen at -18°C to -20°C for up to 120 days if the mixed extraction buffer is less than 14 days old. Samples in Extraction Buffer can be stored at 18-24°C for up to 12 hours;

2-8°C for up to 96 hours; or frozen at -18°C to -20°C for long term storage. Return any unused wells or plates to a sealed amber bag with desiccant pack.

## Required Materials Not Provided with Test Kit

Single channel pipette
Multi-channel pipette
Pipette tips
Sample Vials
Container for mixing extraction
Deionized or Distilled water
Microplate cover lid
Microplate washer (automatic/manual)
Reagent reservoirs
Ear Notcher

## Samples Used for Testing

Bovine ear notches are used for testing. Test results are dependent on the quality of the specimen tested. Improper handling or storage of tissue samples may affect the accuracy of the test results.[5,6]

Samples are taken with a standard pig ear notcher producing a triangular shape of 1.25 cm x 1.25 cm x 0.65 cm.

## Limitations

For detection of BVDV antigen in bovine ear notch samples it is recommended that animals should be >60 days of age due to the potential for interferences of maternal antibody.[7] Not intended to be used with pooled samples as pooling can decrease accuracy.[8] This diagnostic kit cannot type BVDV.

## Testing Recommendations

Rapid in vitro diagnostic for the detection of bovine viral diarrhea virus in persistently infected animals using bovine ear-notch biopsy samples.

## Preparation of Extraction Buffer

Extraction Buffer is prepared by adding the contents of 1 vial of Extraction Buffer Powder to 500 mL of Deionized or Distilled water. Gently mix by hand by inverting and swirling; or mix with magnetic stirrer for 5-10 minutes. Add 350 µL of ProClin 300 and mix again for 5-10 minutes.

## Sample Preparation

Samples are taken with a standard pig ear notcher producing a triangular shape of 1.25 cm x 1.25 cm x 0.65 cm. Place appropriately sized ear notch into sample vial with 1.5 mL of mixed Extraction Buffer.

Allow sample to soak in Extraction Buffer for a minimum of 45 minutes and up to 12 hours at 18-24°C. Ear notches can be stored in Extraction Buffer for up to 96 hours at 2-8°C or frozen at -18℃ to -20℃ for up to 2 years. Longer extraction times will improve the color change of positive samples.

## Precautions

Handle all reagents and samples as biohazardous material.
Wear suitable protective clothing.
Use precaution to not contaminate reagents.
Never pipette by mouth.
Do not use this kit after the expiration date.
Keep out of reach of children.

## Test Procedure

Ensure all reagents and samples have come to 18-24°C before use and ear notch samples have soaked in Extraction Buffer for a minimum of 45 minutes prior to testing.

Remove microwell test plate from package. Use only appropriate number of wells required to test controls and samples. Extra wells should be returned to package and sealed.
Dispense 125 µL of positive control into well A1.
Dispense 125 µL of negative control into well B1.
Dispense 125 µL of samples into wells starting from C1 on.
Dispense 50 µL of Working Conjugate into all test wells.
Cover plate.
Incubate 20 (±3) minutes in the dark at 18-24°C. Wash plate with 350-1,500 µL of Distilled or Deionized water 3 times, by hand or automatic wash machine. Avoid plate drying between plate washings and prior to the addition of TMB Substrate. Tap each plate on an absorbent towel after last wash to remove residual wash.
Dispense 125 µL of TMB Substrate into all wells.
Cover plate.
Incubate 10 minutes in dark at 18-24°C.
Read visually.

### Test Validity

Positive control must have obvious blue color change.
Negative control must not have obvious blue color change.

### Interpretation of Results

Any sample well having obvious color change is considered positive for BVDV antigen in a valid test. While not necessary, immediate retesting following the protocol with this diagnostic kit of all samples with color change will help rule out technician error.

### Follow Up Testing

Any sample resulting in a positive result is considered positive for BVDV antigen. Conclusive diagnosis of BVDV persistent infection (PI) is only determined by follow-up testing of a second sample on this diagnostic kit obtained at least 3 weeks after the initial sample and resulting in a positive result on a valid test.

## References


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8. Cleveland SM, Salman MD, Van Campen H: 2006, Assessment of a bovine viral diarrhea virus antigen capture ELISA and a microtiter virus isolation ELISA using pooled ear notch and serum samples. *J Vet Diagn Invest* 18:395-398.