The Rapid Detection of Bovine Respiratory Syncytial Virus Antigens by Use of a Commercial Enzyme Immunoassay

Sandra J. Rodgers, Charles A. Baldwin
Oklahoma Animal Disease Diagnostic Laboratory
College of Veterinary Medicine,
Oklahoma State University
Stillwater, Oklahoma 74078

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Introduction

Bovine respiratory syncytial virus was isolated in Switzerland in 1970 and was subsequently isolated in the United States in 1974.[1,2,3,4] Numerous animals are exposed to bovine respiratory syncytial virus (BRSV) antigens as antibody levels reported in animals include 81% in Iowa, 74% in Oklahoma, 67% in Alabama, 67% in Minnesota, and 38% in Maryland.[1,2,3,5] Bovine respiratory syncytial virus may predispose animals to secondary infections with other viral or bacterial pathogens.[1,2,3,4,5] Secondary pathogens may include *Pasteurella* spp., *Mycoplasma* spp., *Hemophilus somnus,* infectious bovine rhinotracheitis virus (IBR), bovine viral diarrhea virus (BVD), and parainfluenza 3 virus (PI_3).[1,2,3,5]

Diagnosis of infections with BRSV have previously been limited to histopathologic descriptions of atypical interstitial pneumonia with microscopic lesions of large, multinucleated giant cells and syncytial cells in the lung.[1,4,15] False negative fluorescent antibody (FA) tests on tissue sections may be a result of a viral infection of only superficial bronchial cells.[4] Virus isolation is even a less successful method of identification than FA with a report in one study of 1.7% positive isolations.[6] Most isolates require numerous subpassaging before a cytopathic effect is noticed, but even after numerous passages, the viral titers remain low.[1,2,3,7] Another method for identifying current infections with BRSV is the indirect fluorescent antibody on paired sera to detect a rise in antibody titer.[2,6,8] New rapid assays for diagnosing human respiratory syncytial virus have been compared to the slower and less sensitive conventional methods of serology and viral isolation.[8-12]

The enzyme immunoassays have been found to be a highly sensitive and specific technique for the identification of human respiratory syncytial virus antigens.[9-12] The antigenic similarities between bovine and human respiratory syncytial virus allowed the use of the human assay for the detection of the bovine virus. The Oklahoma Animal Disease Diagnostic Laboratory evaluated the Abbott TestPack® RSV, which requires less than 30 minutes, and compared it to the slower determinations of virology, necropsy, histopathology and bacteriology.

Materials and Methods

Specimens from 69 different bovines with respiratory involvement were submitted to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) from December 1988 through July 1989 for virologic examination. Most bovines (91.3%) were ≤ 1 year of age. Lung and/or organ pool specimens were prepared as 30% w/v homogenates in virologic transport media (Eagles' minimal essential media with Earle's salts containing 0.325% bovine serum albumin and antibiotics). The homogenates were centrifuged at 500 x g for 10 minutes in a table-top centrifuge. An aliquot (0.75 ml) of the supernatant portion was tested for the presence of RSV antigens using the Abbott TestPack®[a] RSV (henceforth referred to as TestPack). All steps were performed at room temperature. The supernatant was transferred to a specimen treatment cup and was treated with 3 drops of specimen treatment buffer, mixed and allowed to incubate for 5 minutes. The specimen was clarified using a filter tube provided in the kit. To the filtered specimen, 3 drops of anti-RSV coated microparticle suspension was added, followed by the addition of 3 drops of anti-RSV biotin. The mixture was gently mixed and allowed to incubate for 10 minutes. The mixture was then poured into the focuser of the reaction device and allowed to soak through the focuser. The focuser was discarded from the reaction device and 3 drops of anti-biotin alkaline phosphatase was added to the device and allowed to incubate for 3 minutes. The reaction device was then washed with a dropperful of guanidine hydrochloride. After complete absorption, 3 drops of chromagen were added. The color reaction was allowed to develop for 2 minutes. The device was then washed with a dropperful of guanidine hydrochloride and the color reaction observed. A positive sign (+) indicated the presence of RSV antigen and a negative sign (-) indicated the absence of RSV antigen.

Viral isolation was attempted on lung tissue and/or organ pools, which may have included lung, liver, spleen and thymus, prepared as 20% w/v homogenates in virologic transport media. The homogenates were centrifuged at

[a]*Abbott Laboratories, Abbott Park, Ill.*

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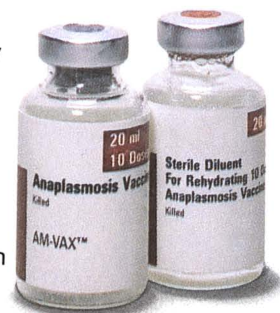

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500 x g for 10 minutes in a table-top centrifuge. The supernatant portion was used to inoculate preformed monolayers of either bovine turbinate cells or Madin-Darby bovine kidney cells by routine virus isolation procedures(2,6,9). Subculture from these specimens was performed twice in flasks and glass chambered microscope slides.[b] Infected chambered-microscope slides were harvested, fixed in acetone for 10 minutes at -20 C and tested for the presence of antigens to IBR virus, BVD virus, BRSV and PI_3 virus using a direct fluorescent antibody test(2,7).

Fluorescent microscopy was performed using frozen lung tissues sectioned at a thichness of 3-4 microns, placed on a microscope slide to air-dry at room temperature and fixed in acetone at -20 C for 10 minutes. The tissue sections were tested for the presence of antigens to IBR virus, BVD virus, BRSV and PI_3 virus using the direct fluorescent antibody test.(2,7) All direct fluorescent antibody test slides were examined for specific immunofluorescence using a microscope equipped with an ultraviolet light source.[c]

Animals or tissue specimens from 54 of the 69 calves were submitted to the OADDL for necropsy and/or histological examination. After necropsy, the tissues were fixed in buffered neutral 10% formalin, paraffin embedded and sectioned at a thickness of 6 microns. The tissue sections were processed and were stained with hematoxylin and eosin using standard histologic procedures.(1,2,4) Mounted slides were examined for pathologic lesions by the use of a light microscope.

Bacteriologic examinations were done on tissues from 49 of the 69 calves using standard techniques(2). In addition, pure cultures of *Pasteurella hemolytica, Pasteurella multocida, Actinomyces pyogenes* and *Hemophilus somnus* were grown in the OADDL bacteriology laboratory. Suspensions of these commonly isolated bovine respiratory bacteria were suspended in distilled-deionized water and tested by the TestPack method, as previously described. Virus suspensions of 4 different strains of BRSV[d] (ATCC, Nebraska, Diamond, and 375L) and other bovine respiratory viruses[d] (i.e., IBR virus, BVD virus, PI_3 virus and bovine coronavirus) were also tested by the TestPack method. The four different strains of BRSV were all positive by the TestPack

All of the bacterial and non-BRSV viral pathogens were determined negative by the TestPack.

[b]*Labtek slides, Nunc, Inc., Naperville, Ill.*

[c]*Leitz Orthoplan Microscope, xenon arc lamp (400HBO), and exciter (KP 500) and Barrier (KP 515) filters, E. Leitz, Inc., Rockleigh, NJ.*

[d]*Provided by the National Veterinary Services Laboratory, Ames, Iowa.*

Results

A total of 69 tissue homogenates were tested by the Abbott TestPack. Of those, 25 (or 36%) of the specimens were positive for antigens to RSV (henceforth referred to as BRSV) (Table 1).

Table 1 Diagnosis of BRSV by the Abbott TestPack® RSV as Compared to Histopathology and Virology

Method	No. Tested	Pos (%)	Neg (%)
TestPack	69	25(36)	44 (64)
Histopathology*	54	43 (80)	11 (20)
Virology	96	6(9)	63 (91)

* Compatible diagnosis was referred to as positive, incompatible diagnosis was referred to as negative.

Histopathologic examination of a total of 54 specimens determined that 43 (or 80%) were compatible with a possible diagnosis of BRSV or a paramyxovirus. Histopathologic lesions seen included acute interstitial pneumonia consisting of eosinophilic, proteinaceous casts within the lining of alveoli, proliferation and sloughing of alveolar cells or the presence of multinucleated cells.

Virologic testing of the 25 TestPack positives produced 6 (24%) that were positive by direct FA testing. None of the 25 samples were positive for BRSV on tissue culture. In addition, 25 of 69 were positive for IBR by direct FA on tissue sections and/or cell cultures, 5 of 60 were positive for BVD by direct FA on tissue sections and/on cell cultures, 1 of 69 was positive for DN599 by direct FA on cell culture and 2 of the 69 were positive for rotavirus by enzyme immunoassay (Table 2).

Other pathogens identified among the test specimens included 19 *Pasteurella* spp. isolated in the TestPack posi-

Table 2 Additional Pathogens Identified in Bovine Respiratory Cases

Identification of Other Pathogens	Abbott Test Pack® RSV Pos	Neg
Pasteurella hemolytica	16	10
Pasteurella multocida	3	5
Actinomyces pyogenes	4	7
Hemophilus somnus	2	2
IBR*	11	14
BVD*	2	3
Rotavirus	1	1
DN599	0	1

* IBR and BVD were identified concurrently from four specimens.

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tive specimens as compared to 15 isolates among the TestPack negative specimens. Additional pathogens identified included: *Actinomyces pyogenes, Hemophilus somnus,* IBR, BVD, rotavirus and DN599 virus (Table 2).

Eighteen (or 82%) of 22 positive specimens were determined to be compatible with a possible diagnosis of BRSV infection by histopathology (Table 3). Tissues remaining from the 3 TestPack positive specimens were not examined histopathologically. Virology procedures determined that only 6 of the 25 specimens to contain BRSV antigens. An agreement in diagnosis of BRSV using the TestPack, histopathology, and virology found only 2 specimens to be positive (by all three methods) out of a total of 55 specimens.

Table 3 Comparison of Various Techniques for the Diagnosis of BRSV

Technique	Abbott TestPack® RSV	
	pos	neg
Pos Histopathoyogy*	18	26
Neg Histopathology*	5	8
Pos Virology	4	3
Neg Virology	20	49
Pos Virology + Pos Histopathology*	2	0
Pos Virology + Neg Histopathology*	1	0
Neg Virology + Pos Histopathology*	16	26
Neg Virology + Neg Histopathology*	3	7

* Compatible diagnosis was referred to as positive, incompatible diagnosis was referred to as negative.

Discussion

Histopathology can be very meaningful in trying to diagnose animals affected with bovine respiratory syncytial virus. However, lesions associated with BRSV can be minimal and associated with many other respiratory disease. The presence of syncytial cells can only be suggestive of an infection with specific viruses, such as BRSV and PI_3 virus, but the presence or absence of such syncytial cell lesions are not diagnostic.[1,4,5] In addition, post-infection with secondary bacterial and viral pathogens are frequent.[1,2,3,5,7]

Use of viral isolation and fluorescent antibody techniques have been shown to produce unsuccessful results with BRSV infections.[1,2,3,13] Several factors combine to cause a very low isolation rate, namely the virus is unstable outside the body and is easily neutralized by a rapidly produced antibody.[4,5,13] In addition, few cell layers are infected with BRSV, allowing limited antigen in tissue sections for detection by fluorescent antibody determinations.

The Abbott TestPack RSV seems to be an easy, rapid, sensitive and specific test to use for the determination of BRSV antigens in bovine tissues. This rapid diagnosis of BRSV (less than 30 minutes) allows immediate treatment to be performed on remaining affected and unaffected bovines, rather than waiting for several days for inconclusive results from histopatholoy, unsuccessful results from virology or several weeks for serologic results.

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