# Blocking ELISA for distinguishing infectious bovine rhinotracheitis virus (IBRV)-infected animals from those vaccinated with a gene-deleted marker vaccine

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## Summary

A sensitive and specific blocking enzyme-linked immunosorbent assay (ELISA) was developed to distinguish infectious bovine rhinotracheitis virus (IBRV)-infected animals from those immunized with a glycoprotein gIII deletion mutant, IBRV(NG)dltkdlgIII. For this ELISA, undiluted test sera are used to block the binding of an anti-IBRV gIII monoclonal antibody (mAbgIII)-horseradish peroxidase (HRPO) conjugate to gIII antigen. TMB substrate is used for color development. Negative S/N values (defined as the absorbance at 650 nm of test sera/absorbance at 650 nm of negative control sera) of $>0.80$ were obtained with immune sera from gnotobiotic cattle immunized with several bovine viruses, with bovine antisera to bovine herpesvirus-2, and vesicular stomatitis virus, with porcine antisera to pseudorabies virus and parvovirus, and with normal sera from heterologous species. Negative S/N values were also obtained with sera from rabbits twice vaccinated with IBRV(NG)dltkdlgIII. However, the S/N values became positive (S/N $<0.8$) 10 to 17 days after the rabbits were challenge exposed to virulent IBRV(Cooper). Most of 116 sera (84%) from feedlot cattle with virus neutralization (VN) titers of $<1:2$ or $<1:4$ had negative S/N values $>0.8$, but 18 sera with negative VN titers had positive S/N values, consistent with observations indicating that an IBRV outbreak was occurring in one of the feedlot herds. Thirty nine sera (98%) from feedlot cattle with VN titers of 1:2 to 1:128 had positive S/N values ($<0.8$). One serum with a VN titer of 1:2 had a borderline ($\pm$) S/N value of 0.81. After immunization with a commercial gIII-

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positive IBRV vaccine, 115/116 sera with VN titers of 1:2 to 1:256 had positive S/N values ( <0.8). One serum with a VN titer of 1:2 had a negative S/N value of 0.83. Serum from one vaccinated animal that failed to seroconvert after vaccination (VN <1:4) showed a strongly positive ELISA S/N of 0.48.

Infections bovine rhinotracheitis virus; Bovine herpesvirus-1; Deletion mutants; Vaccine; Thymidine kinase; Glycoprotein gIII

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## Introduction

The herpesvirus (bovine herpesvirus-1; BHV-1) associated with infectious bovine rhinotracheitis (IBR) is an important pathogen of cattle, which causes severe respiratory infections, conjunctivitis, encephalitis, loss of milk yield, metritis, enteritis, infectious pustular vulvovaginitis/ balanoposthitis (IPV/ IPB), and abortions (Gibbs and Reyemamu, 1977). IBRV (BHV-1) was first isolated in the 1950s. However, it was not until the 1960s, with increasing population stress in livestock production, that the severe respiratory aspects of IBRV infection became increasingly prominent in USA feedlots along with conjunctivitis in calves and abortion storms in in-contact cows. Beginning about 1972, the more severe respiratory form of IBR also became widespread in western European countries (Edwards, 1988; Ackermann et al., 1990a,b,).

Various methods have been used to diagnose and control IBR. In some countries, test and slaughter policies have been followed. In others, modified-live and killed IBRV vaccines have been used to control disease. However, despite the various control programs, IBR has continued to increase. Furthermore, within the European Community, the free traffic of animals will begin in January of 1993. This could further accelerate the spread of disease. Hence, there is an urgent need for better control and eradication programs (Ackermann et al., 1990a,b).

In Switzerland, with a national herd consisting of about 2 000 000 cattle including more than 800 000 breeding animals, a test and slaughter eradication program was initiated 10 years ago. About 50 000 seropositive animals have been slaughtered at a cost of about 110 000 000 Swiss francs (Ackermann, 1990b). The cost of maintaining this program is estimated at an additional 5 000 000 Swiss francs per year. Although appropriate for small countries like Switzerland, it seems unlikely that similar test and slaughter programs would be feasible or cost-effective in countries with much larger cattle herds. Thus, alternative approaches are needed. One such approach now under consideration is that of vaccinating cattle with genetically engineered deletion marker vaccines so as to protect the cattle, and then testing the cattle with differential diagnostic test kits so that infected animals may be distinguished from vaccinated animals and culled from the herd (Kit et al., 1990b). The gene-deleted marker vaccine and differential diagnostic test approach has already

been used in worldwide pseudorabies control and eradication programs (Kit, S. and Kit, M., 1991).

Modified-live IBRV vaccines attenuated through the functional inactivation of the thymidine kinase (TK) gene have been described (Kit, 1988, 1989; Kit et al., 1985, 1986, 1991). In vivo studies have shown that TK-negative IBRV vaccines can protect cattle against clinical signs of disease and suggest that they may be administered safely to pregnant cows (Kit et al., 1986; Miller et al., 1991). It may be noted that extensive field usage had also shown that TK-negative pseudorabies vaccines are efficacious and safe for newborn piglets and for sows in all stages of gestation (Kit, S. and Kit, M., 1991). Starting with the parental TK-deleted IBRV, a second generation IBRV marker vaccine with a deletion mutation in a major IBRV glycoprotein gene, that is, gIII, has now been developed (Kit et al., 1990b). The present study describes a gIII blocking ELISA test for use in conjunction with the gIII gene-deleted IBRV marker vaccine.

ELISA tests for the detection of IBRV have previously been described (Bolton et al., 1981; Collins et al., 1984/1985, 1985). However, to our knowledge, this is the first description of a differential IBRV ELISA test for use with a vaccine marker.

## Materials and Methods

### Coating of wells with antigen

Triton X-100 extracts were prepared from the gIII-positive IBRV(NG)dltk parental virus, as described (Kit et al., 1990a), and diluted 1:1600 (as determined by a titration trial coating process) with KPL (Kirkegaard and Perry Laboratories, Gaithersburg, MD) coating buffer, which contains 0.01 M phosphate-buffered saline. Then, 0.1 ml aliquots were added to wells of 96-well flat-bottomed microtiter plates. The plates were incubated overnight at 4°C, the supernatant fluids were removed, and 0.3 ml KPL blocking solution (which contains 1% bovine serum albumin in phosphate buffer) was added to each well, and the plates were further incubated overnight at 4°C. The supernatant was again dumped and the wells were rinsed three times with TEN buffer, (6.05 g Trizma, 0.38 g $Na_2EDTA$, 8.76 g NaCl in 1 liter of water adjusted to a pH of 7.2). The plates were then tapped dry and stored at −20°C (Kit, M. and Kit, S., 1991). Coating buffer was added to wells designated as blank wells, but IBRV antigen was omitted. Otherwise, the blank wells were processed in the same way as the other wells.

### Description of sera

Gnotobiotic (G) antisera and virus antisera raised in normal calves were supplied by the NVSL-USDA and had the following VN titers: bovine viral diarrhea (Singer)(G), VN − 1:64, and FASN titer (NADL) − 1:1024; bovine

herpes-1, VN – 1:64; bovine herpesvirus-2, IFA titer $\geqslant$ 1:2048; bovine coronavirus(G), IFA titer – 1:2560; vesicular stomatitis (Indiana), VN $\geqslant$ 1:512; vesicular stomatitis (New Jersey), VN $\geqslant$ 1:512; bovine respiratory syncytial virus(G), VN 1:128; and parainfluenza-3(G), IFA titer – 1:2560. The anti-swine PRV, anti-bovine rotovirus, and anti-porcine parvovirus sera were also supplied by the NVSL-USDA (Kit et al., 1990a). Normal lamb, chick, and fetal bovine sera were purchased from GIBCO-BRL (Gaithersburg, MD). Horse serum was purchased from Inovar Biologicals, Gaithersburg, MD. Normal mouse and rabbit sera were obtained from healthy mice and rabbits, respectively. Sera from Texas feedlot cattle were obtained by Dr. Stewart McConnell from Dr. Richard Mock, Texas Veterinary Medical Diagnostic Laboratory, Amarillo, TX. In addition to sera collected at the time the cattle arrived at the feedlot, sera were collected from some feedlot cattle after immunization with a commercial gIII-positive IBRV vaccine.

VN analyses on the bovine sera were carried out at the Texas Veterinary Medical Diagnostic Laboratory. Anti-IRBV gIII blocking ELISA analyses on all sera were carried out at the Baylor College of Medicine in Houston, Texas.

Anti-IBRV rabbit sera was prepared as follows: three young female New Zealand rabbits weighing about 1 kg were pre-bled by ear and then inoculated intramuscularly with about $2 \times 10^3$ PFU of the marker vaccine, IBRV(NG)dltkdlgIII. The animals were observed daily for clinical symptoms of disease (Kelly, 1977). At 14 days postvaccination, sera were obtained and the rabbits were vaccinated again intramuscularly with about $2 \times 10^3$ PFU of IBRV(NG)dltkdlgIII. At 21 days after the second vaccination, the rabbits were again bled and then challenged intramuscularly with about $3.5 \times 10^6$ PFU of the virulent IBRV(Los Angeles) strain. Sera were obtained at 6, 10, 17 and 25 days after challenge. The animals remained healthy after vaccination and challenge. Rabbit sera were heated for 30 min at 58°C, and VN titers were determined by plaque reduction assays as described (Kit et al., 1991).

*ELISA assay procedure*

The procedures used were analogous to those employed previously in the sensitive anti-PRV glycoprotein gIII blocking ELISA for distinguishing between pseudorabies-infected and vaccinated pigs (Kit et al., 1990a; Kit, M. and Kit S., 1991). In each plate, two wells were used as blank wells (lacking antigen), two wells for negative controls (fetal bovine sera lacking IBRV antibodies), and two wells for positive control (sera with IBRV antibodies). Aliquots (0.1 ml) of test sera or of negative and positive control sera were added to appropriate wells of microtiter test plates. A negative serum was added to one blank well and a positive serum to the other blank well. The plates were covered and incubated for 2 h at room temperature (21–25°C), the fluid was removed, and the wells were rinsed three times with wash buffer (50 mM Tris-HCl, pH 7.2, 150 mM NaCl, 1 mM EDTA). IBRV anti-gIII monoclonal antibody(G2) – horseradish peroxidase conjugate (Collins et al., 1984/1985,

1985) was then added to each well. The plates were covered, incubated at room temperature for 30 min, and rinsed five times with wash buffer, with tapping dry between washes. Finally, 0.1 ml of premixed TMB substrate solutions I and II (KPL) were added to all of the wells, the plates were incubated at 21–25°C for 20 min, and then 0.5 ml of a stop solution containing 0.01% sodium azide was added to each well, and the color was analyzed by reading the absorbance at 650 nm. The absorbance ($A$) of the blank well (about 0.05) was subtracted from the $A$ readings of the other wells. The antigen and monoclonal antibody-conjugate concentrations were adjusted so that the $A$ values of the negative control (fetal calf sera) were from 1.0 to 1.5. The $A$ values of the positive control (bovine anti-IBRV sera) were about 0.2 to 0.4. Results on test sera were expressed as S/N values, defined as the $A$ of the test sample (S) divided by the $A$ of the negative control (N).

## Results

### Preliminary control experiments and analyses of gnotobiotic antisera

Anti-IBRV gIII blocking ELISAs carried out on fetal bovine sera, gamma globulin-free fetal bovine sera, and on normal horse, lamb, chick, rabbit, and mouse sera demonstrated that the ratios (S/N) of the $A$ values at 650 nm for these tests samples (S) to the $A$ values at 650 nm of fetal bovine sera (N) were about 0.94 to 1.10 (Table 1). Similarly, bovine antisera to rotovirus, vesicular

TABLE 1

Anti-IBRV-gIII blocking ELISA analyses on gnotobiotic (G) antisera obtained from the national veterinary service laboratories (NVSL) and on additional positive and negative control sera

| Sera | IBRV gIII ELISA (S/N) |
|---|---|
| Anti-bovine diarrhea virus (BVD) (NVSL-G) | 0.86 |
| Anti-IBRV (NVSL) | 0.33 |
| Anti-bovine herpesvirus-2 (NVSL) | 1.15 |
| Anti-bovine coronavirus (NVSL-G) | 1.31 |
| Anti-vesicular stomatitis virus (VSV) (Indiana) (NVSL) | 1.39 |
| Anti-VSV (NJ)(NVSL) | 1.24 |
| Anti-bovine respiratory syncytial virus (NVSL-G) | 1.02 |
| Anti-parainfluenza-3 (NVSL-G) | 1.00 |
| Anti-bovine rotovirus | 1.11 |
| Anti-IBRV (cow No. 14) | 0.35 |
| Anti-IBRV (rabbit) | 0.26 |
| Anti-swine pseudorabies virus (Bartha K) | 0.98 |
| Anti-porcine parvovirus | 1.40 |
| Horse | 0.94 |
| Lamb | 0.95 |
| Chick | 0.95 |
| Fetal bovine | 0.97 |
| Fetal bovine (gamma globulin-free) | 0.95 |
| Mouse | 1.10 |
| Rabbit | 0.96 |

stomatitis virus, bovine herpesvirus-2 and porcine antisera to PRV and parvovirus, exhibited S/N values of 0.98 to 1.40. Sera supplied by the NVSL-USDA and obtained from gnotobiotic (G) calves immunized with several different viruses had S/N values of 0.86 to 1.31, while antisera from calves immunized with IBRV and sera from rabbits infected with IBRV showed S/N values of 0.33, 0.35 and 0.26. On the basis of these empirical results and the analyses to be described, a tentative decision was made to consider S/N values > 0.80 as negative and S/N values < 0.80 as positive for IBRV gIII antibodies. The caveat is, of course, that sera giving S/N values of 0.75 to 0.85 should be considered borderline ( ± ), requiring repetition of assays or assay of additional sera collected from the same animals.

*Analyses on sera from feedlot cattle*

Anti-IBRV gIII blocking ELISAs were carried out on 116 sera collected from feedlot cattle with VN titers of < 1:2 or < 1:4, that is, sera from cattle presumed to be negative for IBRV antibodies (Fig. 1). Most of these sera (84%) had S/N values of > 0.8, consistent with the presumption that they lacked IBRV gIII antibodies. However, 18 of the sera with VN titers of < 1:2 or < 1:4 had S/N values of < 0.8, indicating that these cattle were probably positive for IBRV gIII antibodies.

*IBRV-positive bovine sera*

Anti-IBRV gIII blocking ELISAs were carried out on 40 sera from feedlot cattle showing VN titers of 1:2 to 1:128. (Fig. 2). Thirty-nine of these sera (98%) had positive S/N values of < 0.8. One serum with a VN titer of 1:2 exhibited a borderline ( ± ) S/N values of 0.81.
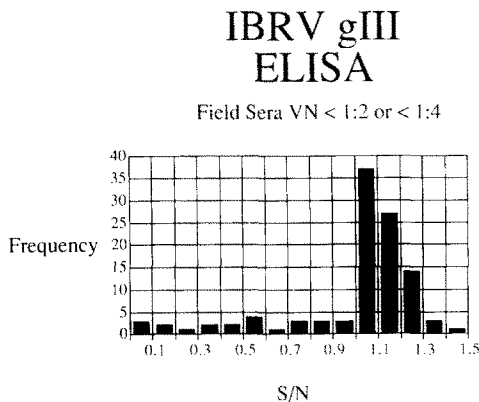
# IBRV gIII
# ELISA

Field Sera VN < 1:2 or < 1:4



Fig. 1. Anti-IBRV gIII ELISA test on 116 samples of feedlot cattle sera with virus neutralization (VN) titers of < 1:2 or < 1:4.

# IBRV gIII
# ELISA
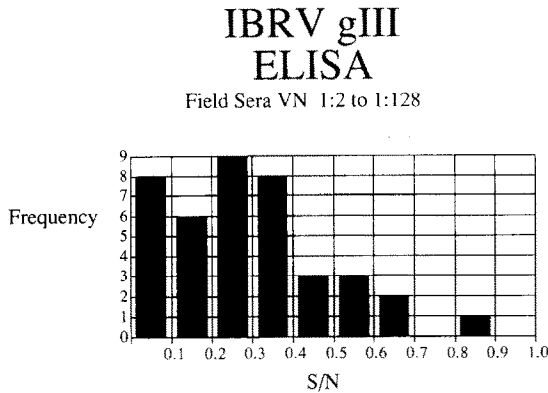Field Sera VN 1:2 to 1:128



Fig. 2. Anti-IBRV gIII ELISA test on 40 samples of sera from feetlot cattle with VN titers ranging from 1:2 to 1:128.

*Sera from feedlot cattle experiencing an IBRV outbreak*

The observations and tests on one herd of cattle (designated No. 901–985 and SMD and RED) were particularly instructive. The 87 cattle of this herd had been trucked into a feedlot and showed some evidence of distress on arrival. Therefore, the cattle were sampled for virus exposure. Sera were collected and 10 were selected at random and bled for buffy coat cultures. Seven of the 10 buffy coat samples were positive for IBRV. The IBRV isolations were verified by VN analyses. Fourteen out of 84 of the sera tested were seropositive by VN conclusively, indicating that this herd was experiencing an IBRV outbreak at the time they arrived at the feedlot. Twenty six (26/84) of the sera analyzed were positive by the anti-IBRV gIII blocking ELISA test (S/N $<0.80$). Of these 26 IBRV gIII-positive sera, 15 had VN titers of $<1:4$ (Table 2) while the other 11 had VN titers of $>1:4$. As a precaution, all calves were vaccinated with an IBRV gIII-positive commercial vaccine. The sera collected 24 days after arrival at the feedlot showed that 80 of the 81 sera tested were positive by VN (1:4 to 1:256). All sera tested (81/81) were positive by the anti-IBRV gIII blocking ELISA test, including a serum (No. 981) negative ($<1:4$) by VN (Table 2). This indicates that IBRV gIII antibodies were induced even though VN antibodies were not detected in serum No. 981.

*Sera from cattle vaccinated with gIII-positive commercial vaccine*

Overall, anti-IBRV gIII blocking ELISA analyses were carried out on 116 sera of cattle vaccinated with a commercial, gIII-positive IBRV vaccine and showing VN titers of 1:2 to 1:256 (Fig. 3). One serum with a VN titer of 1:2 had a borderline negative value of 0.83 but all other sera were positive by the

TABLE 2

Virus neutralization (VN) titers and GIII ELISA analyses on calves trucked to a Texas feedlot and vaccinated with a commercial IBRV vaccine

| Calf No. | Prevaccination | | Postvaccination | |
|---|---|---|---|---|
| | 1/VN | gIII ELISA | 1/VN | gIII ELISA |
| 906 | <4 | 0.31* | 64 | 0.12* |
| 915 | <4 | 0.79 | 64 | 0.29 |
| 918 | <4 | 0.59 | 32 | 0.35 |
| 921 | <4 | 0.25 | NT** | NT** |
| 923 | <4 | 0.51 | 16 | 0.15 |
| 929 | <4 | 0.59 | 128 | 0.03 |
| 938 | <4 | 0.10 | 64 | 0.05 |
| 952 | <4 | 0.30 | 16 | 0.06 |
| 953 | <4 | 0.05 | 64 | 0.02 |
| 958 | <4 | 0.08 | 128 | 0.06 |
| 959 | <4 | 0.38 | 16 | 0.07 |
| 963 | <4 | 0.06 | 16 | 0.07 |
| 965 | <4 | 0.48 | 32 | 0.09 |
| 968 | <4 | 0.13 | 128 | 0.01 |
| 974 | <4 | 0.42 | 16 | 0.62 |
| 981 | <4 | 1.03 | <4 | 0.48 |

*S/N values
**not tested

blocking ELISA test.

*Analyses on sera of rabbits vaccinated with marker vaccine IBRV (NG)dltkdlgIII and challenge exposed to virulent IBRV(Cooper)*

Young New Zealand rabbits immunized with the IBRV(NG)dltkdlgIII marker vaccine developed VN antibodies 14 days after vaccination and showed



# IBRV gIII ELISA
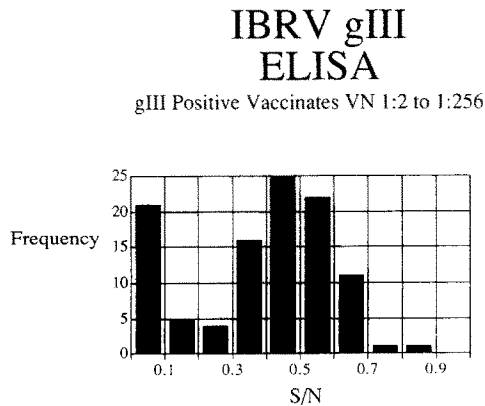gIII Positive Vaccinates VN 1:2 to 1:256

Fig. 3. Anti-IBRV gIII ELISA test on 116 sera from feedlot cattle vaccinated with a commercial gIII-positive vaccine and showing VN titers of 1:2 to 1:256 at 24 days postvaccination.

TABLE 3

Virus neutralization titers and gIII blocking ELISA analyses of sera from rabbits twice vaccinated with IBRV (NG) dltkdlgIII and then challenge exposed to virulent IBRV (Cooper)

| Days after vaccination | Days post challenge | No. 9611 | | No. 9612 | | No. 9613 | |
|---|---|---|---|---|---|---|---|
| | | 1/VN | gIII ELISA | 1/VN | gIII ELISA | 1/VN | gIII ELISA |
| 0 | – | <2 | 1.05[**] | <2 | 1.05[**] | <2 | 0.98[**] |
| 14[*] | – | 4 | 0.84 | 4 | 0.88 | 4 | 1.02 |
| 35 | – | 16 | 0.85 | >128 | 0.87 | 16 | 0.90 |
| 41 | 6 | 128 | 0.81 | >128 | 1.14 | 32 | 1.06 |
| 45 | 10 | 128 | 0.98 | >128 | 0.87 | 32 | 0.65 |
| 52 | 17 | 64 | 0.59 | >128 | 0.63 | 128 | 0.45 |
| 60 | 25 | 64 | 0.39 | >128 | 0.59 | 64 | 0.59 |

[*]Day of second vaccination.
[**]S/N values ($A$ of test samples/$A$ of negative control.

anamnestic responses after a second vaccination and after challenge with the virulent Cooper strain of IBRV (Table 3). Anti-IBRV gIII blocking ELISA analyses showed negative S/N values on pre-vaccination sera and on sera collected after two consecutive vaccinations with the marker vaccine. The S/N values became positive for IBRV gIII antibodies 10–17 days after challenge exposure to IBRV (Cooper).

**Discussion**

The anti-IBRV gIII blocking ELISA is simple, accurate, reproducible, and rapid and was modelled on the highly sensitive and specific glycoprotein gIII blocking ELISA test which distinguishes PRV-infected pigs from pigs vaccinated with a PRV gIII-deleted marker vaccine (Kit, M. and Kit, S., 1991). The IBRV gIII glycoprotein used as antigen in the blocking ELISA test is a major component of IBRV particles. IBRV gIII, like the homologous PRV gIII and HSV gC glycoproteins, has a role in the initial attachment of virus particles to heparin-like receptors on the surface of permissive cells, so that the deletion of gIII probably delays the replication and spread of IBRV in vivo (Kit, S. and Kit, M, 1991; Liang et al., 1991). However like the homologous PRV gIII and HSV gC, the IBRV gIII is not essential for virus replication, indicating that there are alternative mechanisms for virus attachment.

IBRV gIII is classified as a 'late' protein (Ludwig and Letchworth, 1987). This and the fact that it is expressed at high levels on the surface of virus particles and on the surface of virus-infected cells, and also the fact that it is a major target for the induction in cattle of high levels of virus neutralizing antigens makes IBRV gIII an advantageous and reliable marker for a differential diagnostic test (van Drunen Littel-van den Hurk et al., 1990; Hutchings et al., 1990). The high level of induction of virus-neutralizing antibodies and the importance of gIII for differential diagnostic testing

counterbalances any reduction in protection of cattle that might occur through the use of IBRV gIII deletion mutant as vaccines. It may be noted that even in the absence of gIII, several important immunogens capable of inducing protection are available. For example: (i) IBRV glycoproteins gI and gIV, the homologues of the essential HSV glycoproteins gB and gD, respectively, are major antigens involved in the induction of IBRV neutralizing antigens early in infection (Ludwig and Letchworth, 1987); (ii) IBRV gIV appear to be the antigen which most consistently stimulates the proliferation of lymphocytes from IBRV-immunized animals (Hutching et al., 1990); (iii) the activation of natural killer cell activity against IBRV-infected cells does not require late viral glycoproteins, like gIII (Cook et al., 1989; Palmer et al., 1990); (iv) protective immune responses are obtained by injection of individual IBRV glycoproteins gI, gIII, and gIV, with gIV immunization providing the best individual protection (Babiuk et al., 1987; van Drunen Littel-van den Hurk et al., 1990) and (v) essential glycoprotein gH (Meyer et al., 1991) and other IBRV proteins (e.g. VP8) may contribute to the induction of protective immune responses. In the case of the complex herpes viruses, there is neither theoretical nor empirical evidence that deletion of the nonessential gIII(gC) glycoprotein impairs the protective response (Kit, S. and Kit, M., 1991).

The anti-IBRV gIII blocking ELISA utilizes undiluted test sera. Hence, time consuming and laborious dilutions of test sera prior to addition to the antigen-coated wells is not required. The gIII blocking ELISA is specific, as may be seen from the observations that high titered gnotobiotic antisera to heterologous viruses, normal sera from heterologous species, and antisera to the pseudorabies herpesvirus and to bovine herpesvirus-2 gave negative S/N values. Likewise, sera from animals twice vaccinated with the gIII deletion mutant, IBRV (NG)dltkdlgIII, exhibited negative S/N values.

The sensitivity of the anti-IBRV gIII blocking ELISA is demonstrated by the detection of gIII antibodies in the sera of VN-negative ( < 1:4) cattle in a herd experiencing an IBRV outbreak, and by the detection of gIII antibodies in 115/116 calves vaccinated with a gIII-positive commercial vaccine. The one serum from the vaccinate that showed a false negative had a VN titer of 1:2 and a ( $\pm$ ) S/N of 0.83. Another calf that failed to seroconvert by VN ( < 1:4) had a strongly positive S/N of 0.48. In addition, all the VN-positive (VN = 1:2 to 1:128) field sera tested were gIII ELISA-positive, except for one serum with a VN = 2, which showed an S/N of 0.81, close to the borderline cut-off value separating negative from positive sera.

Finally, the observation that gIII antibodies were detected in sera of animals twice vaccinated with the marker vaccine, IBRV(NG)dltkdlgIII, by 10 to 17 days postvaccination suggest that the gIII blocking ELISA test is sufficiently sensitive to detect those field strain infections of vaccinated cattle which have the potential to reactivate from latency and spread in a herd. The development of the gene-deleted IBRV marker vaccine and differential diagnostic test kit may facilitate the implementation of IBRV control/eradication programs analogous to those already undertaken for Aujeszky's disease.

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