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Antigen-capture blocking enzyme-linked immunosorbent assay based on a baculovirus recombinant antigen to differentiate *Transmissible gastroenteritis virus* from Porcine respiratory coronavirus antibodies

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Abstract. A new commercially available antigen-capture, blocking enzyme-linked immunosorbent assay (antigen-capture b-ELISA), based on baculovirus truncated-S recombinant protein of *Transmissible gastroenteritis virus* (TGEV) and 3 specific monoclonal antibodies, was developed and evaluated by examining a panel of 453 positive Porcine respiratory coronavirus (PRCoV), 31 positive TGEV, and 126 negative field sera by using another commercially available differential coronavirus b-ELISA as the reference technique to differentiate TGEV- from PRCoV-induced antibodies. The recombinant S protein–based ELISA appeared to be 100% sensitive for TGEV and PRCoV detection and highly specific for TGEV and PRCoV detection (100% and 92.06%, respectively), when qualitative results (positive or negative) were compared with those of the reference technique. In variability experiments, the ELISA gave consistent results when the same serum was evaluated on different wells and different plates. These results indicated that truncated recombinant S protein is a suitable alternative to the complete virus as antigen in ELISA assays. The use of recombinant S protein as antigen offers great advantages because it is an easy-to-produce, easy-to-standardize, noninfectious antigen that does not require further purification or concentration. Those advantages represent an important improvement for antigen preparation, in comparison with other assays in which an inactivated virus from mammalian cell cultures is used.

Key words: Antigen-capture blocking enzyme-linked immunosorbent assay; Porcine respiratory coronavirus; recombinant S protein; *Transmissible gastroenteritis virus*.

Introduction

Transmissible gastroenteritis virus (TGEV; family *Coronaviridae*, genus *Coronavirus*) causes a highly contagious enteric disease of swine characterized by vomiting, severe watery diarrhea, and a high mortality in piglets less than 2 weeks of age. However, in herds with endemic transmissible gastroenteritis (TGE), the majority of piglets are unaffected, which makes the clinical identification of these endemically infected herds difficult.[32] To detect such carrier animals, a simple and reliable diagnostic method for monitoring the status of TGEV infection in herds was required.

Transmissible gastroenteritis virus was first isolated in 1946.[8,10] In 1984, a nonenteropathogenic virus related to TGEV, Porcine respiratory coronavirus (PRCoV), appeared in Europe[27] and later in North America.[47] In contrast to TGEV, PRCoV causes a mild subclinical respiratory infection.[27,47] Until recently, a virus neutralization (VN) assay was used to evaluate antibodies to TGEV. However, antibodies to TGEV and PRCoV cannot be distinguished by using classic methods such as VN, indirect enzyme-linked immunosorbent assay (ELISA), or fluorescent antibody tests,[2,13] which constitutes an important problem for the diagnosis of the disease.[40,42,45,47]

Three major structural proteins were described for coronaviruses: spike glycoprotein (S; 180–200 kDa), membrane (M; 21–30 kDa), and nucleoprotein (N; 45–50 kDa)[20,23,30,31,46] (Fig. 1), the S protein being the most interesting from the antigenic and immunogenic points of view.[9,22,41,42] Four antigenic sites, mapped on the S protein in the order C, B, D, and A, starting from the N-terminal end, were recognized on the S protein.[6,7,11,14] Antibodies to those antigenic sites can be found in the serum of TGEV-infected pigs. The absence of 2 of these antigenic sites (B and C) in the S protein of PRCoV as a consequence of the deletion of 224–227 amino acids, was the basis for their differentiation from the enteric viruses.[28,29,33,34] A number of serologic ELISAs based on monoclonal antibodies (mAb) were developed to differentiate TGEV and PRCoV.[1,3,18,35,37,43] However, to the authors' knowledge, to date, there have been only 2

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