

<b>Short Report</b>
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## USE OF A RECOMBINANT FOOT-AND-MOUTH DISEASE VIRUS 3D POLYMERASE IN AN AGAROSE GEL IMMUNODIFFUSION TEST

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*In the present study, examination was made of the effectiveness of a complete recombinant 3D polypeptide in an AGID test (AGID-3D), for use in the detection of FMDV-infection-specific antibodies, regardless of vaccination condition. Results indicate that compared with the traditional AGID VIAA, the AGID 3D offers, particularly when assessing low titer sera, a more consistent method, with comparable specificity, and at least equal sensitivity. Neither of the antigens offered any particular advantage with regard to band differentiation. Replacement of the VIAA by a recombinant 3D antigen has considerable attractions, since it provides an unlimited supply of a safe, inexpensive, easily purified and consistent material, eliminating the potential presence of non-specific BHK or capsidial antigens.*

The term “virus infection associated antigen” (VIAA) of foot-and-mouth disease virus (FMDV) conventionally refers to a complex of non-structural proteins identified by Cowan and Graves (5), the major component of which is the viral RNA polymerase, 3D (13).

The first reported technique for detecting antibodies against VIAA as an aid to indicate previous FMDV infection was the agar gel immunodiffusion test (AGID) (8). Due to the low sensitivity of this assay, Alonso collaborators (3), developed a liquid-phase enzyme-linked immunosorbent assay (ELISA-VIAA) to identify and quantify antibodies against FMDV-VIAA. However, the improved sensitivity attained by applying this kind of assay, when compared with the AGID-VIAA,

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increased the number of VIAA-positive results in cattle vaccinated and reimmunized with vaccines containing high concentrations of non-purified FMDV antigens. Moreover, limited reproducibility of the VIAA tests(6) (I. Bergmann personal communication) appears to be an inherent property of the semi-purified nature of the VIAA preparations, which are subjected to variation in antigen quality among different batches. Furthermore, for production of conventional antigen, the FMDV-VIAA is partially purified from virus suspensions produced in baby hamster kidney (BHK) cells, which requires a high-security laboratory unit for handling FMDV (1,9).

To overcome the mentioned limitations, the replacement of the VIAA by a recombinant FMDV polymerase (3D) antigen has considerable attractions, since it provides an unlimited supply of a safe, inexpensive, easily purified and consistent material. Moreover this approach eliminates the potential presence of non-specific antigens, like BHK or capsidial antigen components in the VIAA preparations, which may be recognized in the AGID-VIAA test by sera from cattle inoculated with BHK-produced vaccines, leading to false-positive results. In the present study, examination was made of the effectiveness of a complete recombinant 3D polypeptide, constructed and purified as described previously (10), in an AGID test (AGID-3D), for use in detection of FMDV-infection-specific antibodies, regardless of vaccination condition.

A comparative study of the use of the traditional VIAA and the recombinant 3D polypeptide in an AGID test indicated that neither of the antigens offered any particular advantage with regard to band differentiation.

No difference in the positions, intensity, rate of formation and visibility of the precipitation bands was observed.

High specificity of the AGID-3D test was indicated by negative results for 250 sera from cattle in aphthovirus-free areas. Thus, high specificity of the AGID-VIAA was maintained when using the recombinant antigen.

AGID-3D-positive results were obtained when sera from 60 cattle involved in field outbreaks, bled 20-40 days after detection of the disease, were assessed. Therefore, no difference with the overall sensitivity of the AGID-VIAA test could be established. Titration of the reactive sera in the AGID-3D gave values 1-3 times higher than those obtained in the AGID-VIAA, depending on the VIAA preparation. Follow-up of sequentially collected sera from 4 persistently infected cattle indicated that irrespective of the 3D batch, AGID-3D detected antibodies indicative of viral replication for up to about 280 days after infection. Such stage of the infection could be detected with only one of the four VIAA preparations studied. These findings clearly indicate the increased consistency of the AGID results obtained with the recombinant 3D antigen when compared to those yielded by the traditional VIAA preparation, particularly when assessing low-titer sera.

Further studies were conducted to determine whether the AGID-3D could eliminate positive results yielded by AGID-VIAA for post-vaccination sera (2). To this effect, the reactivity of sera with an unexpectedly high number of AGID-VIAA-positive results was examined. Samples from systematically vaccinated cattle in regions with no FMD for at least the past 3 years, yet

showing AGID-VIAA-positive results in 61% (147/240) of the sera obtained from cattle > 2 years old, and in 11% (22/207) of those collected from the < 2 years old cattle, born after the last FMD outbreak, were studied. Of the AGID-VIAA-positive sera in the population over and under than 2 years of age, 91% and 41%, respectively, also reacted with the recombinant 3D. Overall positivity was reduced to 55% and 4% in the > and < 2 years old cattle, respectively. Similarly, reactivity of sera with unusually high number of AGID-VIAA-positive results (78 of 90 cattle) obtained 30 days after a single dose of vaccine had been administered 120 days after initial vaccination was assessed. Vaccine antigens were obtained in roller flasks, and in contrast to standard production procedures, these antigens were not clarified; also, they were concentrated fourfold. Of the 78 VIAA-positive sera, 7 became negative in the AGID-3D. Also analyzed were experimental sera from two groups of 20 cattle, each vaccinated and revaccinated twice at 60-day intervals, with vaccines prepared with antigens produced in suspension cell cultures and concentrated to 18 and 54 µg/5ml dose were also analyzed. Most of the cattle, which were AGID-VIAA-positive between 15 and 30 days after revaccination, were also reactive by AGID-3D.

These data clearly indicated that a number of AGID-VIAA-false-positive results in post-vaccinated sera were eliminated by AGID-3D. In most cases, however, such reactivities were maintained and likely represented anti-3D antibodies elicited against the RNA polymerase present in the vaccine preparations. In fact, early findings by Rowlands and collaborators (14) showed that antiserum produced in guinea pigs that had received highly purified inactivated

virus particles reacted with VIA antigen isolated from virus. More recent evidence by electron microscopy indicated that the 3D protein is a component of at least 20-30% of the virus particles (11). Failure to detect reactivity against 3D in many vaccinated animals may be related to insufficient concentration of the 3D polypeptide in the viral suspensions, as well as to the relatively low sensitivity of the AGID test. In this context, the same recombinant 3D applied in immunoenzymatic tests (10), clearly showed that antibodies against 3D are present in sera from many vaccinated cattle. Such reactivities become particularly evident after revaccination. O'Donnell and collaborators (12), using another bacterially expressed 3D recombinant protein established anti-3D antibody-positive results transiently in sera from cattle immunized with vaccines from one out of the two producers tested. Taking into account: a) the observations mentioned above; b) that methods for antigen purification and concentration used to manufacture vaccines in laboratories in South America may vary widely; c) that under field conditions high immunological coverage is expected in areas with advanced eradication programs; and d) that stimulation of antibodies upon immunization can be affected by animal age, boosters from previous infections and vaccination cycles, the 3D/VIAA should be used with caution, at least, when highly sensitive assays are required.

Great progress has been made to distinguish unequivocally in a field setting infected from non-infected animals, regardless of their vaccination condition, by the use of additional bioengineered non-capsidial viral antigens, other than 3D, in an EITB assay (4), or

ELISA tests (7). In spite of the high sensitivity, the tests eliminated a large number of VIAA/3D-AGID-positive results due to vaccination and revaccination. Maximum sensitivity could be maintained under the assumption that the simultaneous assessment of many antigens will resolve the consequent loss of specificity. Because of the high sensitivity and specificity, these assays allow confirmation of the absence of FMDV activity as required by the Office International des Epizooties for international recognition as being FMD-free. The application of such sensitive tools is also relevant during the eradication process prior to suspension of vaccination and as an input in risk analysis for import/export testing.

Although for the latter purposes, the AGID test lacks sufficient sensitivity, it is still a useful tool in many laboratories, as an aid in periodical surveys to determine the prevalence and incidence of antibodies against VIAA/3D in the animal population susceptible to the disease, and to establish whether a herd has or has not had recent contact with the FMD virus. For such cases the use of a recombinant antigen guarantees biosafety, and compared with the traditional AGID VIAA, offers a more consistent method, with comparable specificity, and at least equal sensitivity.

## REFERENCES

1. Alonso Fernández A, Sondahl MS. Preparación y concentración de los antígenos 140 S, 12 S y VIA del virus de la fiebre aftosa. *Bol Cent Panamerican Fiebre Aftosa* 1975; (17-18): 1-6.
2. Alonso Fernández A, Gomes I, Bahnemann H. The induction of antibodies against VIAA in cattle vaccinated and revaccinated with inactivated foot-and-mouth disease vaccine. *Bol Cent Panamerican Fiebre Aftosa* 1988; (54): 43-50.
3. Alonso Fernández A, Gomes MPD, Martins MA, Sondahl MS. Detection of foot-and-mouth disease virus infection associated antigen antibodies comparison of the enzyme-linked immunosorbent assay and agar gel immunodiffusion test. *Prev Vet Med* 1990; 9: 233-240.
4. Bergmann IE, Augé de Mello P, Neitzert E, Beck E, Gomes I. Diagnosis of persistent aphthovirus infection and its differentiation from vaccination response in cattle by use of enzyme-linked immunoelectrotransfer blot analysis with bioengineered non-structural viral antigens. *Am J Vet Res* 1993; 6:825-841.
5. Cowan KM, Graves JG. A third antigenic component associated with FMD infection. *Virology* 1966; 30: 528-540.
6. Mackay DKJ, Madekurozwa RL. Assessment of the VIAA ELISA for FMD. Report for the Community Reference Laboratory for FMD. Pirbright: Institute for Animal Health; 1992.
7. Mackay DKJ, Forsyth M, Davies PR, Berlinzani A, Belsham GJ, Flint M, Ryan MD. Differentiating infection from vaccination in foot-and-mouth disease using a panel of recombinant non-structural proteins in ELISA. *Vaccine* 1994; 16:446-459.
8. McVicar JW, Suttmoller P. Foot-and-mouth disease: the agar gel diffusion precipitin test for antibody to virus infection-associated (VIA) antigen as a tool for epizootiologic surveys. *Am J Epidemiol* 1970; 92:273-278.
9. Morgan DO, Moore DM, McKercher PD. Purification of foot-and-mouth disease virus infection-associated antigen. In: *Proceedings Eighty-Second Annual Meeting of the United States Animal Health Association*; 1978 Oct 29-31, Nov 1-3, Buffalo, New York. Richmond, Virginia: USAHA; 1978. pp. 277-283.
10. Neitzert E, Beck E, Augé de Mello P, Gomes I, Bergmann IE. Expression of the aphthovirus RNA polymerase gene in *E. coli* and its use together with other bioengineered non-structural antigens in detection of late persistent infections. *Virology* 1991; 184:799-804.

11. Newman JFE, Piatti PG, Gorman BM, Burrage TG, Ryan MD, Flint M, Brown F. Foot-and-mouth disease virus particles contain replicase protein 3D. *Proc Natl Acad Sci USA* 1994; 91:733-737.
  12. O'Donnell VK, Boyle DB, Sproat K, Fondevila AF, Schudel AA, Smitsaart EN. Detection of antibodies against foot-and-mouth disease virus using a liquid-phase blocking sandwich ELISA (LPBE) with a bioengineered 3D protein. *J Vet Diagn Invest* 1996; 8: 143-150.
  13. Polatnik J, Arlinghaus RA. Foot-and-mouth disease virus-induced RNA polymerase in baby hamster cells. *Virology* 1967; 31: 601-608
  14. Rowlands DJ, Cartwright B, Brown F. Evidence for an internal antigen in foot-and-mouth disease virus. *J. Gen Virol* 1969; 4:479-487.
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## RESUMO

### **Uso de uma proteína recombinante do vírus da febre aftosa, a polimerasa 3D, em uma prova de imunodifusão em gel de agar.**

Neste estudo, se examinou a efetividade do uso do polipeptídico 3D recombinante, obtido em sua forma nativa numa prova de IDGA (IDGA-3D), para uso na detecção de anticorpos específicos de infecção com VFA, independentemente da condição de vacinação. Os resultados indicam que em relação à prova tradicional de IDGA-VIAA, a IDGA 3D oferece,

particularmente quando se avaliam soros de baixo título, um método mais consistente, com especificidade comparável e pelo menos a mesma sensibilidade. Nenhum dos antígenos ofereceu uma vantagem particular com respeito à definição das bandas de precipitação. A substituição do VIAA pela proteína 3D recombinante tem consideráveis atrações, dado que proporciona um fornecimento ilimitado de material inócuo, econômico, de fácil purificação e consistente, eliminando a presença potencial de antígenos não específicos de células BHK ou componentes do capsídeo do VFA.