Production and Evaluation of A Standard Diagnostic Rinderpest Antigen

Afaf A. Abdel-Wahab[1] and Saad,M.A.A[2]

1- Vet. Serum and Vaccine Res. Institute;
2-Central Lab. for Eval. of Vet. Products,
Abbassia, Cairo

Abstract

A specific rinderpest antigen was produced on the basis of standard operating procedures using the attenuated rinderpest virus (RBoK) and Vero cells. Quality control tests used were agar gel immunodiffusion test, direct immunofluoresence and immune-capture ELISA. Results of these tests for the prepared antigen were comparable to those for the reference control rinderpest antigen.

Introduction

Rapid, accurate diagnosis is an essential precursor for success in disease surveillance (**Anderson et al., 1996**). Detection of antibody to rinderpest (RP) is a reliable tool for the diagnosis of RP and the demonstration of using RP antibody titre is a reliable indicator of recent infection (**1; 2; 3; 5; 6**). Hence, the availability of a standardized RP antigen is indispensible as a tool that should be at hand in time of need. Virtually every diagnostic and research oriented test in virology begins with an antigen that has been specially prepared for that test. So, the aim of the present work was to produce and evaluate a RP specific diagnostic antigen that can be effectively and reliably used in rinderpest sero-diagnostic purposes.

Material and Methods

1-Rinderpest virus (RPV):

An attenuated RPV, strain RBoK (**15**), adapted on vero cells was supplied by Rinderpest Research and Vaccine Production Department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. It had a titre of 10^6 $TCID_{50}$/ml. This virus strain was used to prepare RPV antigen.

2-African Green Monkey kidney Cell Culture (Vero cells):

Vero cells (monkey, African green), American Type Culture Collection, ATCC, No. CCL-81, originally established by (**17**). Vero cells were used for preparation of RP antigen.

3-The production of Rinderpest antigen:

The attenuated RP virus, strain RBoK (**15**) was grown in cultures of Vero cells. When full cytopathic effects (CPE) have spread through the cell sheets, the monolayers were then washed three times with warm phosphate buffered saline (PBS) and the cells were scraped from the glass, suspended in a small volume of PBS and such infected cells were subjected, thereafter, to freezing and thawing several times (5-6 times) at -20°C and room temperature, respectively. The virus suspension was subsequently concentrated by dialysis and the concentrated suspension was followed by clarification through two successive centrifugations, low speed centrifugation (2000 rpm for 30 minutes at 4°C) then ultra centrifugation (10000 rpm for 10 minutes at 4°C). The obtained supernatant was used as an antigen (**8**).

4-Reference anti-rinderpest hyperimmune sera:

Standard anti-rinderpest hyperimmune serum was used to evaluate the prepared antigen using agar gel immunodiffusion test while Rinderpest hyperimmune serum conjugated with fluorescin isothiocynate, horseradish peroxidase used for the same purpose using FAT and immunocapture ELISA. These kits were supplied from CIRAD/IEMVT, 10 Pierre Curie, 94704 Maisons-Alfort, Paris, France. Reference positive as well as negative controls were included in the test.

5-Evaluation of the produced rinderpest antigen:

5.1- Sterility test:

The produced antigen was cultured for aerobic/anaerobic bacteria on soya been Casein Digest and thioglycollate respective media, for mycoplasma on M-CMRL medium and for fungi on Sabouraud's medium according to standard operating procedures (**14**).

5.2- Purity:

The antigen was re-passed (undiluted and 10 fold serial dilutions up to 10^{-5}) on vero cells, observed daily for 10 days for cytopathic effects (CPE) according to (**11**).

5.3- Efficacy:

5.3.1-Agar gel immunodiffusion test (AGID):

This test was done as described by (**16**). The produced antigen was tested against specific reference antibody, reference rinderpest antigen/antibody reaction was included in the test.

5.3.2-Direct fluorescent antibody technique (FAT):

The monoclonal antibody based direct immune-fluorescence test was used according to the method described by (**4; 7; 9**). The test included reference positive and negative controls.

5.3.3- Immuno capture enzyme linked immunosorbent assay (ELISA):

This test was carried out according to the method described by (**4; 10**).

Results

1-Sterility and purity:

The produced rinderpest antigen was found to be free from aerobic/anaerobic bacteria, mycoplasma as well as fungi. It was found to be free from residual rinderpest virus as of negativity of vero cells to CPE.

2-Efficacy:

The produced rinderpest antigen was found to be efficacious as specific reactions were observed with AGID, direct immunofluorescence and immunocapture ELISA, as follows:

2-1- Agar gel immunodiffusion test (AGIDT):

The tested antigen produced sharp precipitation lines in reaction to the reference rinderpest antibody comparable to reference reaction (Photo 1).

2-2- Direct immunofluorescence:

Immunofluorescence was detected with the tested antigen, comparable to the reference positive rinderpest antigen (Photo 2).

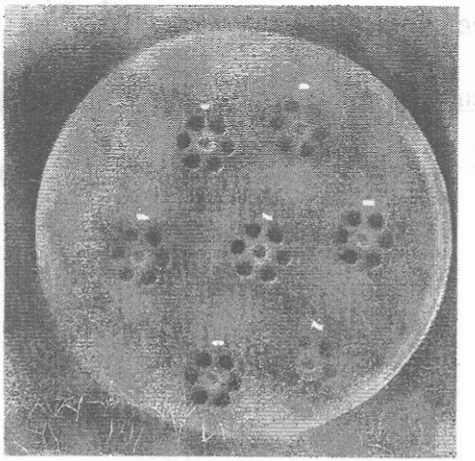

Photo (1) showing sharp precipitin lines in reaction

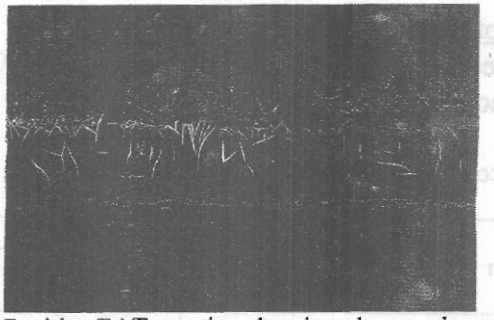

Photo (2) Positive FAT reaction showing clear apple green spots

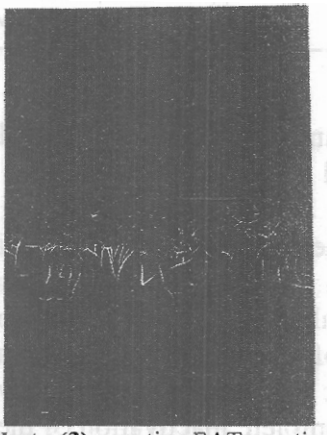

Photo (3) negative FAT reaction

3-3- Immunocapture ELISA:

The tested antigen produced OD value of 0.935 comparable to that given by reference positive rinderpest antigen (0.933).

Table (1) Quality control of the prepared rinderpest antigen

Tested antigen	Applied tests		
	AGID	Direct FAT	Immunocapture ELISA
The prepared antigen	4 +ve clear sharp precipitation line	4 +ve	OD = 0.935
Positive control reference	4 +ve clear sharp precipitation line	4 +ve	OD = 0.933
Negative control reference	-ve	-ve	0.03

Discussion

Based on standard operating procedures and good laboratory practices, the obtained results revealed that the prepared specific rinderpest antigen was found to be sterile as evidenced through the use of specific growth media for aerobic/anaerobic bacteria, mycoplasma and fungi. Beside, it was proven pure, free from any residual rinderpest virus. These two criteria are crucial for the safe handling of the antigen as well as for avoiding the liability of spreeding infection of laboratory material (**13**)

It was evident that the process of several cycles of freezing and thawing resulted in complete inactivation of the virus without damaging the antigenic components of the virus. This result as well as the efficacy of the antigen were confirmed through the application of direct immunofluorescence (Photo 1), agar gel immunodiffusion test and immunocapture ELISA. The reactions observed with the three techniques as they are of strict specificity were good proofs on the validity of the product.

Although these three techniques (AGID, FAT and ELISA) were used to evaluate the prepared rinderpest antigen; however, the latter test could be considered the test of choice for field application. This is based on the grounds of emergency need to test sera samples collected from large

numbers of animals to obtain accurate results of serodiagnosis (**12**). The prepared rinderpest antigen might be thus used, replacing the high cost commercial product, for rinderpest serosurvillence (**2; 3; 5**).

The result obtained with the AGID test was an additional proof on the good quality of the produced antigen, as it produced sharp clear precipitation line with the reference rinderpest antibody comparable to the reference antigen/antibody reaction (**16**).

References

1. Anderson,J. (1984): "Use of monoclonal antibody in a blocking ELISA to detect group specific antibodies to blue tongue virus". J.Imm.Meth., 74: 139-149.
2. Anderson,J. and McKay,J.A. (1994): "The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes". Epidemiol.Infect., 112: 225-231.
3. Anderson,J. and Rowe,L.W. (1982): "Application of enzyme immuno-assays in rinderpest research". In R.C.Wardley and J.R.Crowrher, eds. The ELISA: Enzyme-Linked immunosorbent assay in veterinary reaserch and diagnosis, p. 176-191. The Hague, The Netherlands, Martinus Nijhoff.
4. Anderson,J.; Barrett,T. and Scott,G.R. (1996): "Manual on the diagnosis of rinderpest". 2nd edition, FAO Animal Health Manual, No.1, FAO of the UN, Rome, 1996.
5. Anderson,J.; McKay,T.A. and Butcher,R.N. (1991): "The use of monoclonal antibodies in competitive ELISA for the detection of antibodies to rinderpest and peste des petits ruminants viruses". In the sero-monitoring of rinderpest throughout Africa, Phase one. Proceedings of the Final Research Coordination Meeting of the IAEA-rinderpest Control Projects. Cote d'Ivoire, November 1999. IAEA publication TECDOC-623. Vienna, Austria. International Atomic Energy Agency (IAEA).
6. Anderson,J.; Rowe,L.W.; Taylor,W.P. and Crowther,J.R. (1982): "An enzyme linked immunosorbent assay for the detection of IgG, IgA and IgM antibodies to rinderpest virus in experimentally infected cattle". Res.Vet.Sci., 32: 242-247.
7. Diallo,A.; Barrett,T.; Barbron,M.; Subborad,S.D. and Taylor,W.P. (1989): "Differentiation of rinderpest and peste des petits ruminants viruses using specific cDNA clones". J.Virol.Meth., 23: 127-136.
8. Hierholzer,J.C.; Killington,R.A. and Stokes,A. (1996): "Preparation of antigens". Chapter 3 in virology methods manual, edited by: Brian,W.J.M. and Hillar,O.K.; Harcourt Brace and Co.Publishers, New York, pp. 47-53.
9. Libeau,G. and Lefevre,P.C. (1990): "Comparison of rinderpest and peste des petits ruminants viruses using anti-nucleoprotein monoclonal antibodies". Vet.Microbiol., 25: 1-16.

10. Libeau,G.; Diallo,A.; Colas,F. and Guerre,L. (1994): "Rapid differential diagnosis of rinderpest and PPR using an immuno-capture ELISA". Vet.Record, 134(12): 300.
11. Obi,T.U. and Patrick,D. (1984): "The detection of peste des petits ruminants virus antigen by agar gel precipitation test and counter immunoelectrophoresis". J. of Hygiene, 93:579-586.
12. OIE (2000): "Manual of standards for diagnostic tests and vaccines". 4th ed., 2000.
13. Rositer,P.B. and Mushi,E.Z. (1980): "Rapid detection of rinderpest virus antigen by counter immunoelectrophoresis". Trop. Anim. Health Prod., 12: 209-216.
14. Rweyemamu,M.M.; Sylla,D.; Palya,V. and Prandota,J. (1991): "Standard operating procedures for rinderpest cell culture vaccine". Quality Control Testing, FAO (1991).
15. Scott,G.R. (1981): "Rinderpest". In J.W.Davies, L.H.Kasstod and D.D.Trainer, eds. Infectious diseases of wild mammals, 2nd ed., Ames, IA, USA, Iowa University Press.
16. Scott,G.R.; Taylor,W.P. and Rossiter,P.B. (1986): "Manual on the diagnosis of rinderpest". FAO animal production and health series No. 23.
17. Yasumura,Y. and Kawatika,Y. (1963): "Studies on SV40 virus in tissue culture". Nibon Rinsho, 21: 1201-1215.

إنتاج وتقييم أنتيجين قياسي لتشخيص الطاعون البقري

عفاف عبد العظيم عبد الوهاب ١ -- محمد أحمد أحمد سعد ٢

١- معهد بحوث الأمصال واللقاحات البيطرية

٢- المعمل المركزي للرقابة على المستحضرات الحيوية البيطرية

العباسية – القاهرة

تم تحضير أنتيجين الطاعون البقري بإستخدام عترة فيروس الطاعون البقري "RBoK" وخلايا فيرو وأجريت ثلاثة إختبارات لتقييمه وهى الوميض الفلورسنتي المناعي وإختبار الإنزيم المرتبط الممرض المناعي الصائد وإختبار الترسيب المناعي في الأجار، مقارنة مع أنتيجين الطاعون البقري المرجعي، وقد ثبت من إستقراء النتائج صلاحية الأنتيجين المحضر للإستخدام خاصة في أعمال المسوح السيرولوجية Serosurvillance.