



## RESEARCH ARTICLE

### Comparison of IFA and ELISA in the Detection of Avian Leukosis Virus Subgroup J in DF-1 Cell Cultures

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#### ABSTRACT

To evaluate three kinds of ELISA avian leukosis virus (ALV) p27 antigen kits A, B, C, an infectious clone rNX0101 of ALV subgroup J were inoculated into DF1 cells with three different doses ( $9 \times 10^3$ ,  $9 \times 10^2$ , 90TCID<sub>50</sub> separately) and the supernatant of DF1 cell cultures were obtained at 1-6 days post inoculation and tested with three kinds of kits after freezing and thawing once. The infected cell was detected by ALV-J specific monoclonal antibody JE9 at 3 and 6 days post inoculation separately also. Results indicated the infected cells at 3 days and 6 days post inoculation all can be identified by IFA after inoculated with different dose, while only the kit A can detect the p27 antigen at 3 days post inoculation with the high dose and none of inoculated cells was detected positively if with B and C ELISA kits at the same time. All samples infected by different doses showed positive to ALV-J whether by three ELISA kits or by IFA 6 days post inoculation. The result indicated the sensitivity between A, B and C ELISA kits in the detection of ALV-J with three doses of inoculation and the IFA is more sensitive than commercial ELISA kit.

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#### INTRODUCTION

Avian leukosis virus (ALV) is a common avian retrovirus associated with neoplastic diseases, exogenous ALV are classified into A, B, C, D, and J subgroups based on their host range, cross-neutralization and viral interference, they can induce different pathotypes of neoplastic diseases in chickens (Witter *et al.*, 2003). Among these subgroups, ALV subgroup J (ALV-J) is more common than other subgroups in chickens and usually isolated and reported in China (Cui *et al.*, 2003; Xu *et al.*, 2004; Lai *et al.*, 2011).

ALV-J was first isolated in 1988 from meat-type chickens in Great Britain (Payne *et al.*, 1991), it mainly induced myelocytomatosis and nephromas in meat-type chickens. During the last 10 years, ALV-J has been reported in many areas of the world (Cui *et al.*, 2003; Malkinson *et al.*, 2004; Thapa *et al.*, 2004; Lai *et al.*, 2011). ALV-J caused losses and spread primarily in a vertical transmission, as there is no commercial vaccine to control ALV-J up to now some breeder farms have to take different eradication measures to eliminate ALV-J.

Usually the blood samples from breeder chickens were cultured in DF-1 cells for 9-14 days and the

supernatant was tested the p27 antigen of ALV by enzymes-linked immunosorbent assay tests (ELISA) using different commercial ALV p27 antigen test kit to identify the exogenous ALV and the positive chickens were eliminated. This procedure has been as the standard procedure in eradication program of ALV-J all over the world. While this procedure highly dependent on the sensitivity and specificity of the commercial p27 detection kit. In this study the sensitivity and specificity of three ALV p27 antigen test kits from China and abroad was compared and confirmed by Indirect immunofluorescence assay (IFA).

#### MATERIALS AND METHODS

##### Cells and Virus

The DF-1 cell lines were purchased from ATCC of US and used for replication of exogenous ALV. DMEM pH7.2 GIBCO, USA was used as the basic medium (containing 100 U/mL penicillin and 100 µg/mL streptomycin), 10% of fetal bovine serum (FBS) was added in the basic medium as the growth medium. For the maintaining medium, only 2% of FBS was added. Cell cultures were kept in an incubator with 5% CO<sub>2</sub> at 37.

The ALV-J field strain NX0101 was first isolated from a meat-type parent breeder farm by our lab in Ningxia province of China in 2001 Cui *et al.*, 2003, its infectious clone rNX0101 was constructed and stored in our lab (Zhang *et al.*, 2005). The infectious clone rNX0101 was amplified, titered and reserved in -80 freezer for further studies.

#### Preparation of different samples infected by different dose of rNX0101

DF-1 cells from a petri dishes was digested and seeded evenly to two 6-well plates and the plate were marked as plate A and plate B separately, one cover glass was prepared in every well for the detection of IFA. When monolayers were formed, the well A1 and A2 in plate A was inoculated with 90ul rNX0101 ( $9 \times 10^3$  TCID<sub>50</sub>) respectively while the wells B1 and B2 in plate B was added 90ul DMEM as negative control. The wells A3 and A4 in plate A was inoculated with 90ul eluted rNX0101 ( $9 \times 10^2$  TCID<sub>50</sub>) separately while B3 and B4 in plate B was added 90ul DMEM as negative control. Similarly the wells A5 and A6 were inoculated with 90 TCID<sub>50</sub> rNX0101 respectively and the well B5 and B6 as negative control. 900ul supernatant of each well was collected at 1d, 2d, 3d, 4d, 5d, 6d post inoculation and stored in -20 freezer. The same volume maintaining medium was supplemented after the supernatant was collected each time. The cover glass from each well was fixed at 3d and 6d after inoculation for the detection of IFA.

#### Detection of different samples by IFA and ELISA

The supernatant from each well was tested the p27 antigen of ALV by ELISA using three different ALV antigen test kit A, B and C according to the manufacturer's instructions. To ensure the accuracy of results, the procedure above was repeated and each sample was tested twice. The ALV-J infection was confirmed by IFA with ALV-J specific monoclonal antibody JE9 according to Qin introduced Qin *et al.*, 2001 . Binding of the primary antibody was detected using FITC-labeled anti-mouse IgG (SIGMA, USA).

## RESULTS

#### Detection with 3 different ELISA kits aimed to p27 antigen

The kit A can detect the p27 antigen at 3 days after inoculated with  $9 \times 10^3$  TCID<sub>50</sub> rNX0101 while the kit B and kit C can detect the p27 antigen at least 4 days after inoculated with the same dose. When the DF-1 inoculated with  $9 \times 10^2$  TCID<sub>50</sub> and 90 TCID<sub>50</sub> rNX0101 separately the p27 antigen can be detected 4 days after inoculation by Kit A and the detection time is delayed one day for Kit B and Kit C. This indicated the Kit A is more sensitive than Kit B and Kit C.

#### Detection with specific monoclonal antibody by IFA

The infected cells at 3 days after inoculated with different dose all can be identified by ALV-J specific monoclonal antibody JE9 and the ratios for positive cells infected by ALV-J corresponding well with the inoculated dose. 6 days after inoculated with different dose all cells

show positive to ALV-J infection and no dramatically difference between them was found.

#### Comparison of IFA and ELISA in the detection of ALV- J

The infection was identified at 3 days after inoculation with different dose by IFA while can only be identified by Kit A at the high dose at the same time. This indicated the IFA is more sensitive than ELISA. The infection was identified at 6 days after inoculation with different dose by 3 ELISA kits and IFA and no significant difference between them was found (Table 1).

**Table 1:** Comparison of IFA and ELISA in the detection of ALV- J in DF-1 cell culture

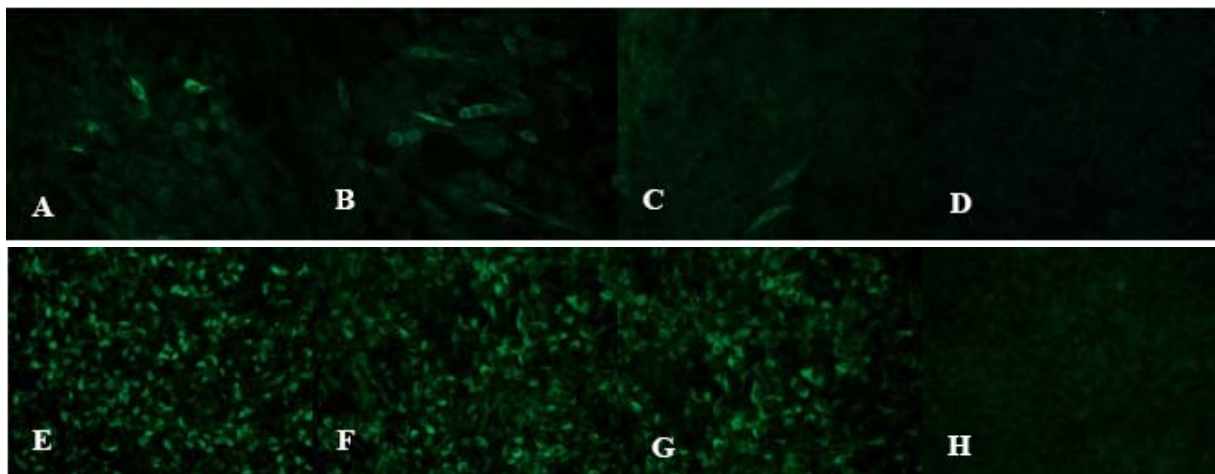
Inoculate dose	Detected by	Days after inoculated with rNX0101					
		1d	2d	3d	4d	5d	6d
$9 \times 10^3$ TCID <sub>50</sub>	ELISA Kit A	—	—	+	+	+	+
	ELISA Kit B	—	—	—	+	+	+
	ELISA Kit C	—	—	—	+	+	+
	IFA	ND	ND	++	ND	ND	++++
$9 \times 10^2$ TCID <sub>50</sub>	ELISA Kit A	—	—	—	+	+	+
	ELISA Kit B	—	—	—	—	+	+
	ELISA Kit C	—	—	—	—	+	+
	IFA	ND	ND	++	ND	ND	++++
90 TCID <sub>50</sub>	ELISA Kit A	—	—	—	+	+	+
	ELISA Kit B	—	—	—	—	+	+
	ELISA Kit C	—	—	—	—	+	+
	IFA	ND	ND	+	ND	ND	++++

Note: "ND" means not done; "—" indicate the result is negative; "+" indicate the detection result is positive and different number of "+" correlated with ratio of the infected cell by rNX0101.

## DISCUSSION

Marek's disease virus (MDV), Reticuloendotheliosis virus (REV) and ALV are three tumor viruses in chickens, all of them could induce different symptoms from subclinical infection to growth retardation, immune suppression and tumors, and always it is indistinguishable from other infections with similar symptoms and made diagnosis more difficult in the field. Among these viruses, ALV-J is more common than other virus in chickens and usually isolated and reported in China in these years (Cui *et al.*, 2003; Xu *et al.*, 2004; Lai *et al.*, 2011). In the field, it causes both late-onset myeloid tumors and acute tumors even in 4-to-5-wk-old birds (Fadly *et al.*, 1999; Payne *et al.*, 1992). So the existence of ALV-J infection in the field might be a long-term problem for the poultry industry in China.

Some breeder farms have taken different eradication measures to eliminate ALV-J, and the ALV-J infection is on the decline in some commercial breeders. To detect the ALV infection, different methods have been developed and promoted for many years, such as virus isolation, IFA and ELISA (Payne *et al.*, 1993; Spencer *et al.*, 1984). Commercial ELISA kit aimed to antigen is usually the first choice for the identification of ALV in the poultry industry but it is dependent on the group specific antigen p27, which is shared by both exogenous and endogenous ALV (Chesters *et al.*, 2002). While as our previous study the S/P value corresponding well with the 50% tissue culture infectious dose (TCID<sub>50</sub>) and the TCID<sub>50</sub> can be



**Fig. 1:** Detection with specific monoclonal antibody by IFA

A.DF-1 cell cultured for 3 days after inoculated with  $9 \times 10^3$  TCID<sub>50</sub> rNX0101; B.DF-1 cell cultured for 3 days after inoculated with  $9 \times 10^2$  TCID<sub>50</sub> rNX0101; C.DF-1 cell cultured for 3 days after inoculated with 90 TCID<sub>50</sub> rNX0101; D.DF-1 cell cultured for 3 days with no rNX0101 inoculated as blank control; E.DF-1 cell cultured for 6 days after inoculated with  $9 \times 10^3$  TCID<sub>50</sub> rNX0101; F.DF-1 cell cultured for 6 days after inoculated with  $9 \times 10^2$  TCID<sub>50</sub> rNX0101; G.DF-1 cell cultured for 6 days after inoculated with 90 TCID<sub>50</sub> rNX0101; H.DF-1 cell cultured for 6 days with no rNX0101 inoculated as blank control

predicted by detecting the p27 antigen S/P value when rNX0101 was cultured in DF-1 cells (Dong *et al.*, 2011). In our lab the dynamic detection with three ELISA Kits to different subgroups of exosomic ALVs in DF-1 Cells was compared previously and the results showed the sensitivity for three ELISA Kits is different from each other and the infection dose is one of the key factors effect testing time (Guo *et al.*, 2010). In this study we also found the sensitivity for three ELISA Kits are different and the Kit A is the most sensitive and can shorten the detection time at least for 1 day. Of course the specificity of three kits is credible and their specificity was confirmed by ALV-J specific monoclonal antibody JE9.

The difference between IFA and ELISA was also compared in this study and we found the IFA is more sensitive than ELISA. When DF-1 cell was inoculated with rNX0101 at a very small dose the p27 antigen can be detected at least 4 or 5 days after inoculation while only 3 days the infection was confirmed by IFA. This indicated the IFA is more sensitive than ELISA when ALV-J cultured in DF-1 cell and IFA can significantly shorten the detection time. From this study it can be concluded that the IFA is more suitable for the rapid diagnosis of some samples. However, when the number of samples is especially huge, ELISA is more suitable than IFA such as in the epidemiological investigation. If these two kinds of method used combine together the sensitivity and specificity in the detection of ALV-J will be greatly enhanced.

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