*Short Communication*

# Detection of subgroup J avian leukosis virus gene by polymerase chain reaction from whole blood without DNA extraction

**Kun Qian, Bolin Hang, Haiyu Shen, Wenjie Jin and Aijian Qin***

Ministry of Education Key Lab for Avian Preventive Medicine and Key Lab of Jiangsu Preventive Veterinary Medicine, College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu 225009, China.

**Avian leukosis virus subgroup J (ALV-J) is an exogenous avian leukosis virus that causes significant economic losses. In this study, a whole blood polymerase chain reaction (PCR) assay without DNA extraction was developed, optimized, and was compared with conventional methods for the detection of ALV-J infection. The evaluation assay indicated that the whole blood PCR had similar sensitivity and specificity when compared with virus isolation and conventional PCR. Taken together, the whole blood PCR method was very specific, easy to perform, rapid and of low cost, which will be useful for routine diagnosis of ALV-J, especially for large scale sampling.**

**Key words:** Avian leukosis virus subgroup J, polymerase chain reaction, whole blood.

## INTRODUCTION

In recent years, avian leukosis virus subgroup J (ALV-J) has become less and less prevalent in white meat-type chickens owing to continued eradication programs implemented by the major international breeding companies. However, myelocytomatosis induced by ALV-J still occurs in white meat-type chickens, commercial layer flocks and local Chinese breeds. And, the mixed structure of the chicken breeding industry in China makes disease control difficult (Cui et al., 2009). Due to lack of effective treatment and vaccine protection, these eradication programs have to depend on epidemiological information and accurate detection of chickens infected with ALVs for the diagnosis of ALV-J infection. Several methods have already been developed and used for both laboratory and field samples in virus detection (Qin et al., 2001; Kim et al., 2002; Li et al., 2007; Zhang et al., 2010; Qiu et al., 2011). Although these techniques offer effective testing, they still require multiple steps with tedious labor and/or expensive kits. In addition, impurity of the final nucleic acid and sample misidentification or contamination with other nucleic acids still remains concerns. In this short communication, we reported to develop as well as evaluate a whole blood PCR assay without viral DNA purification for the detection of ALV-J infection.

### MATERIALS AND METHODS

We tried different volumes of anticoagulant blood and standard Taq DNA polymerase to establish and optimize the whole blood PCR reaction without any buffer system treatment. The blood template was used at 1, 2 and 3 µl, combined with 5 units of Taq DNA polymerase, to determine the best combination of the two factors. Subsequently, we added 5, 4, 3, 2, 1, 0.5, 0.2 and 0.1 units of Taq DNA polymerase with 1 µl positive whole blood template to the PCR reaction to determine the amount of Taq DNA polymerase in the reaction. The results in Figure 1 demonstrated that whole blood is a good template for the PCR, and that 2 units Taq DNA polymerase is sufficient to amplify the target gene from 1 µl of different positive blood samples using the whole blood PCR, although there are numerous inhibitors of PCR in whole blood (Al-Soud and Radstrom, 2001). Thus, we established the whole blood PCR system and procedure as follows: All blood samples were collected into tubes containing the anticoagulant sodium citrate and were stored at 4, −20 or −70°C for further use. For each reaction, the 50 µl PCR system contained 5 µl 10×PCR buffer (without $Mg^{2+}$; Fermentas,

*Corresponding author. E-mail: aijian@yzu.edu.cn. Tel: 86-514-87979224. Fax: 86-514-87979217.
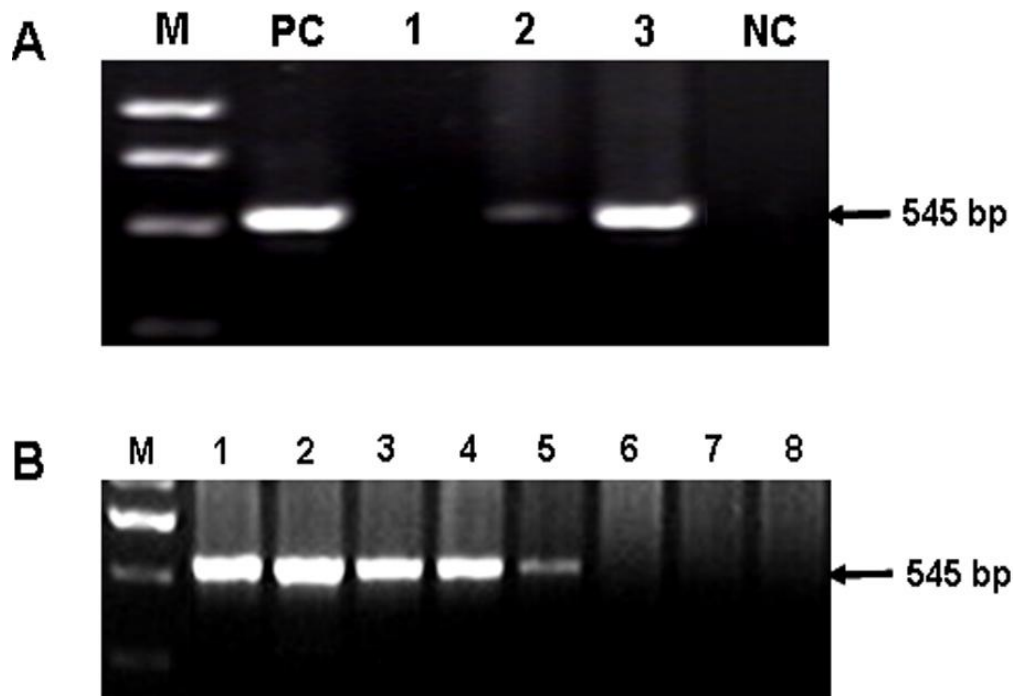
**Figure 1.** Determination of the optimal conditions for the whole blood PCR. (A) The whole blood PCR results obtained with different volume of blood sample. Lanes 3, 2 and 1: 3, 2 and 1 µl of blood as template in 50 µl of PCR reaction; 5 units standard Taq polymerase used for PCR. (B) Optimization of the units of Taq DNA polymerase in the PCR: 5, 4, 3, 2, 1, 0.5, 0.2 and 0.1 units of standard Taq DNA polymerase was added from lane 1 to 8 in 50 µl of PCR reaction with 1 µl positive whole blood template. The total DNA from DF1 cells infected with ALV-J isolate was used as positive control (PC), and the blood or DNA extracted from blood from SPF chickens as negative control (NC). DNA marker was DL2000 from Takara, Dalian China.

USA), 1.5 mM magnesium chloride ($MgCl_2$; Fermentas, USA), 0.15 mM of each of the four deoxynucleotide triphosphates (dNTPs; TaKaRa Biotechnology Co., Ltd, China), 2 units of standard Taq DNA polymerase, 0.25 pM of H5/H7 primer pair which was reported before (Smith et al., 1998), 1 µl of whole blood was used as a template for the whole blood PCR reaction. A touchdown PCR was performed in an ABI 9700 with an initial denaturation step of 5 min at 95°C followed by 13 cycles of denaturation at 93°C for 1 min, annealing at 60°C for 1 min, decreasing by 1°C in each cycle and extension at 72°C for 1 min 30 s, then followed by 30 cycles of 93°C for 1 min, 48°C for 1 min, 72°C for 1 min 30 s, with a final extension at 72°C for 10 min. Subsequently, we confirmed the results of optimized whole blood PCR in ALV-J infection detection by the conventional PCR and sequence. In addition, positive blood samples stored at different temperatures, 4, –20 or –70°C, could be amplified also. There is evidence indicating that the positive blood samples stored at 4°C could still be available for virus detection at least one week. And at least one month while at –20 or –70°C.

To determine the sensitivity of the whole blood PCR assay, 10-fold serial dilutions (from $10^{-1}$ to $10^{-4}$) of blood were used for the whole blood PCR. The agarose gel electrophoresis showed that the standard Taq DNA polymerase was able to detect the template at $10^{-1}$ dilution. Because we do not know the initial virus concentration in the sample blood, this sensitivity assay is a relative measure of sensitivity. Although the sensitivity may not be comparable with that of real-time quantitative PCR, this technique has its own advantages in the detection of ALV-J infection, especially with large scale sampling.

## RESULTS AND DISCUSSION

Both the whole blood PCR and the conventional PCR were validated by being compared to the "gold standard"-virus isolation confirmed by IFA. The protocol of conventional PCR and virus isolation could follow the previous report (Smith et al., 1998; Qin et al., 2001). The 692 whole blood samples, collected from two large-scale chicken farms in Anhui and Jiangsu Provinces, were Hyline brown commercial chicken blood with same age. 38 of these whole blood samples were positive and 647 were negative by the three assay. Seven of the negative samples in virus isolation were positive in whole blood PCR, but only two of them were positive in the conventional PCR. The data analysis in Table 1 revealed that the sensitivities of both PCR platforms were 100% (38/38). The specificity of the conventional PCR was 99.7% (652/654), while 98.9% (647/654) in the whole blood PCR when compared with virus isolation. And, the P value of the estimated agreement between the two PCR was 0.575 by student t test (Table 1). There was no significant difference between the whole blood PCR and the conventional PCR. However, the whole blood PCR (45/692) was a little more sensitive than the conventional

**Table 1.** Comparison of the whole blood PCR with conventional methods for detection of ALV-J from clinical samples.

| PCR | | Virus isolation and IFA positive (38) | Virus isolation and IFA negative (654) |
|---|---|---|---|
| Conventional PCR | Positive | 38[a] | 2 |
| | Negative | 0 | 652[b] |
| Whole blood PCR | Positive | 38 | 7 |
| | Negative | 0 | 647 |

a: Both sensitivities of two PCR platforms were 100% (38/38); b: The specificity of conventional PCR was 99.7% (652/654), while 98.9% (647/654) in whole blood PCR when compared with virus isolation. And the P value of the estimated agreement between the two PCR was 0.575 (>0.05).

PCR (40/692) in clinical samples, since we confirmed the extra five positive samples in the whole blood PCR by sequencing PCR products. The different detection ratio may be related to the reaction of mechanisms of the different detection methods. The evaluation test data indicated that the whole blood PCR without DNA extraction is more efficient than the conventional methods in virus detection.

## Conclusion

Overall, the whole blood PCR assay established in current study is sensitive, rapid, high detection efficiency, and easy to perform, compared with the conventional methods. It also could be modified and applied to various avian diseases that involve viremia, and it would be a useful diagnostic method for the study of virology and pathogenesis, especially for large-scale molecular epidemiological study in the future.

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