

## Different vectors used to transform and clone of nonstructural NS1 gene of Influenza B in *Escherichia coli*

A.A. Dawood

Department of Anatomy, College of Medicine, University of Mosul, Mosul, Iraq  
aliadeldawood@gmail.com

(Received November 9, 2018; Accepted December 21, 2018)

### Abstract

Flu is a highly contagious and common illness caused by influenza A, B, and C viruses. The aim of the present study was to investigate the transformation and cloning of NS1B gene with pET-32a, pET-32b and pQE-81L in *Escherichia coli* BL21(DE3) and DH5 $\alpha$ . pUC57-NS1B synthetic gene was transform and clone in *Escherichia coli* BL21(DE3). Isolation, single digestion and ligation with pET-32b using HindIII restriction enzyme. Amplification of recombinant DNA was done with conventional PCR after transformation. Screening with IPTG of colonies. Gel electrophoresis was done for each step of cloning after isolation. Isolation, double digestion and ligation with pET-32a and pQE-81L using SacI, PstI and HindIII respectively. Recombinant DNA was attempted to be transformed into *E. coli* strains BL21 (DE3) and DH5 $\alpha$ . pUC57 plasmid carrying NS1B gene was successful transformed and isolated from *E. coli* BL21 (DE3). Designed primers used for PCR of NS1B showed successful amplification. First screening of pET-32b-NS1B colonies using white/blue method, cloning NS1B into pET-32b using single restriction digestion with HindIII, pET-32a using double restriction digestion with SacI and HindIII and pQE-81L using double restriction digestion with PstI and HindIII gave unexpected result. This result may relate to re-ligation of digested vector for single digestion and uncompleted digestion for vectors of double restriction digestion. Current study has suggested that recombinant NS1B gene can be cloned using single digestion with other expression vectors.

**Keyword:** Nonstructural protein, Influenza B, Polymerase chain reaction  
Available online at <http://www.vetmedmosul.com>

### استخدام ناقلات مختلفة لنقل واستنساخ الجين غير التركيبي NS1 لفيروس الإنفلونزا ب في بكتريا الايشريكية القولونية

علي عادل داود

فرع التشريح، كلية الطب، جامعة الموصل، الموصل، العراق

### الخلاصة

يعتبر مرض الأنفلونزا مرضًا شائعًا ومُعْدٍ بسبب فيروسات الأنفلونزا نوع أ و ب و سي. كان الهدف من هذه الدراسة هو البحث في تحويل واستنساخ الجين الغير تركيبى NS1B مع النواقل pET-32a، و pET-32b و pQE-81L في بكتريا الايشريكية القولونية نوع BL21 DE3 و DH5 $\alpha$ . تم نقل الجين الغير تركيبى مع حامله pUC57-NS1B الاصطناعي وتم استنساخه في بكتريا الايشريكية القولونية نوع BL21 DE3. تم عزل وقطع الجين بإنزيم واحد وربطه مع الحامل pET-32b باستخدام أنزيم تقبيد HindIII. تم إجراء تضخيم الحمض النووي المؤتلف باستخدام تقنية تفاعل البلمرة المتسلسل PCR التقليدي بعد النقل. تم الفحص باستخدام IPTG للمستعمرات البكتيرية. تمت عملية الترحيل الكهربائي للهلام لكل خطوة من خطوات عملية الاستنساخ بعد العزل. تم عزل وهضم الجين وربطه مع النواقل pET-32a و pQE-81L باستخدام أنزيمات التقبيد SacI، PstI و HindIII على التوالي. تم محاولة ربط ونقل الحمض النووي المؤتلف إلى سلالات بكتريا الايشريكية القولونية نوع BL21 (DE3) و DH5 $\alpha$ . تم تنقل البلازميد pUC57 الحامل للجين الغير تركيبى NS1B وعزله بنجاح في بكتريا الايشريكية القولونية نوع BL21 (DE3). وأظهرت الباءات المصممة المستخدمة لتفاعلات البلمرة المتسلسل PCR في تضخيم الجين الغير

تركيب NS1B نجاحا. أول كشف لمستعمرات البكتريا الحاملة ل pET-32b-NS1B باستخدام طريقة الأبيض / الأزرق. استنساخ الجين الغير تركيب NS1B وربطه مع الناقل pET-32b باستخدام هضم الإنزيم المقيد الأحادي نوع HindIII، ومع الناقل pET-32a باستخدام عملية الهضم المزدوج باستخدام إنزيمي التقييد SacI و HindIII ومع الناقل pQE-81L باستخدام عملية الهضم المزدوج مع إنزيمي القطع HindIII و PstI أعطت نتيجة غير متوقعة. قد ترتبط هذه النتيجة بإعادة ربط الناقل على نفسه المهضوم بانزيم التقييد الاحادي والهضم غير المكتمل للنواقل ذات الهضم المزدوج. اقترحت الدراسة الحالية أنه يمكن استنساخ الجين الغير تركيب NS1B المؤتلف باستخدام الهضم المفرد مع نواقل التعبير الأخرى.

## Introduction

Influenza B virus is an envelope virus, belonging to the family Orthomyxoviridae. Common cold is caused by influenza B and is milder than type A virus. However, some cases lead to severe diseases but rarely morbidity (1). Influenza B viruses lead to seasonal epidemics due to mutations in the viral surface glycoproteins. The influenza nonstructural B protein comprises of 281 amino acids (2). NS1 protein persists only in the infected animal cells and not in vaccinated animals (3). Hence, this may path the way of utilizing NS1 protein as a target for diagnostic of influenza viruses. NS1 protein is involved in virulence and inhibition of the  $\alpha/\beta$  interferon system during virus infection. It was shown that, the expression of the NS1B protein complements the growth of influenza A virus with NS1A deleted (4). NS1 gene from various influenza viruses had been cloned. This gene was successfully expressed in *Escherichia coli* (5). This study describes transformation and cloning of NS1B gene of human influenza B virus in *E. coli* BL21 (DE3) and DH5 $\alpha$  using three types of vectors.

## Materials and methods

All restriction enzymes, DNA polymerase were purchased from Promega. Ligase enzymes were obtained from Yeastern Biotech. Primers used for PCR amplification were synthesized by 1st Base Laboratories, Kuala Lumpur, Malaysia. Plasmid miniprep and gel extraction kits were purchased from GeneAll and QIAGEN. The pET and pQE vectors were ordered from Novagen and QIAGEN, respectively.

Transformation pUC57-NS1B plasmid into *E. coli* BL21 (DE3) competent cells was cultured overnight in Luria Bertani (LB) broth containing 100  $\mu\text{g}/\text{ml}$  ampicillin at 37°C with shaking at 200 rpm. The plasmids were isolated using plasmid miniprep kit from GeneAll- South Korea. The products were detected using gel electrophoresis (BIO-RAD) and viewed on a UV transilluminator (GeneFlash) (6). Single restriction digestion was done for pUC57-NS1B and pET-32b by using HindIII enzyme which recognizes 5'A $\nabla$ AGCTT3' sequence. The DNA concentration and purity were measured by NanoDrop Spectrophotometer from (NanoDrop Technologies) using 1  $\mu\text{l}$  of the sample. NS1B and digested pET-32b

with HindIII were ligated using conventional PCR (Biocompare) (Table 1).

Table 1: Single digestion components in PCR tube using HindIII

pUC57-NS1B	16.8 $\mu\text{l}$	pET-32b	16.8 $\mu\text{l}$
Acetylated BSA	0.2 $\mu\text{l}$	Acetylated BSA	0.2 $\mu\text{l}$
Buffer E	2 $\mu\text{l}$	Buffer E	2 $\mu\text{l}$
HindIII enzyme	1 $\mu\text{l}$	HindIII enzyme	1 $\mu\text{l}$
Total	20 $\mu\text{l}$		20 $\mu\text{l}$

The PCR process was initiated with initial denaturation step at 94 °C for 5 minutes. The process continued with the annealing at 50 °C for 30 second and extension step at 72 °C for 7 minutes. The PCR products were run on gel electrophoresis, then purified by gel extraction (QIAGEN gel extraction kit) to remove the buffer and restriction enzymes. The products were detected using gel electrophoresis. The same protocol was used to isolate pQE-81L from *E. coli* DH5 $\alpha$ . NS1B amplified gene and pQE-81L were digested using PstI and HindIII restriction enzymes (7) (Table 2).

Table 2: Double digestion components in PCR tube using PstI and HindIII

pQE-81L or NS1B gene	15.8 $\mu\text{l}$
Acetylated BSA	0.2 $\mu\text{l}$
10X Multicore enzyme buffer	2 $\mu\text{l}$
HindIII enzyme	1 $\mu\text{l}$
PstI enzyme	1 $\mu\text{l}$
Total	20 $\mu\text{l}$

PstI enzyme recognizes the 5 'TGCA $\nabla$ G3' sequence and HindIII enzyme recognizes 5'A $\nabla$ AGCTT 3' sequence. After transformation, colony PCR was carried out with pQEfor (5'- CGGATAACAATTTACACAG-3') and pQErev (5'- TTCTGAGGTCATTACTGG-3') primers. Chelex 100 resin is highly purified, and most suitable for DNA applications was used for detecting bacteria colonies. With the same protocol, pET-32a was digested with SacI and HindIII restriction enzymes for the third attempt (Table 3).

Table 3: Double digestion components in PCR tube using *SacI* and *HindIII*

pET-32a or NS1B gene	15.8µl
Acetylated BSA	0.2µl
10X Multicore enzyme buffer	2µl
<i>HindIII</i> enzyme	1µl
<i>SacI</i> enzyme	1µl
Total	20µl

PCR amplification was done on pUC57-NS1B to amplify NS1B gene with a newly designed primers NS1Bfor*SacI* (5'-GAGCTCATGGCGAACAAT-3') and NS1Brev*HindIII* (5'-AAGCTTCTAATTGTCTCC-3') which introduced *SacI* and *HindIII* restriction sites to NS1B for directional cloning of the gene into pET-32a. The *HindIII* enzyme recognizes 5' A▼AGCTT3' sequence while *SacI* enzyme recognizes 5' GAGCT▼C 3' sequence. The gene was extracted, double digested with *SacI* and *HindIII* and used for cloning into pET-32a. pET-32a vector's universal primers, namely T7 promoter (5'-TAATACGACTCACTATAGGG-3') and T7 terminator (5'-GCTAGTTATTGCTCAGCGG-3'). The whole investigated clones were 72 clones used in our study for all vectors.

### Results

Transformation of pUC57-NS1B synthetic gene constructs into competent *E. coli* BL21 (DE3) was successfully done. The presence of pUC57-NS1B was verified by agarose gel electrophoresis. The restriction digestion of pUC57-NS1B was successful and NS1B gene (870 bp) was separated and this is shown in Figure 1. pET-32b was also linearized and its presence was verified by the band of 5.9kb in Figure 1.

Double digestion was done for pQE-81L by using *PstI* and *HindIII* enzymes. As for pUC57-NS1B, it was first digested with *HindIII* enzyme and the result is shown in Figure 2.

Isolation and digestion of the vector and gene. Lane 1 and 2 shows the successful digestion of pUC57-NS1B with *HindIII* enzyme and the band of NS1B is of correct size (870bp). Lane 4 shows the double digestion of pQE-81L by using *PstI* and *HindIII* and the band is of correct size (4.8kb) but in this way still unknown whether the digestion correct complete or not (Figure 2). Plasmid miniprep for both pUC57- NS1B and pQE-81L were verified in lane 3 and 5. However, all the colonies screened gave negative result (Figure 3). The size of amplified band is smaller than 300bp, indicating that only the pQE-81L vector was transformed to cells without the cloning of NS1B gene.

The right band size to be expected is nearly 1000bp. The amplified NS1B gene is shown in figure 4.

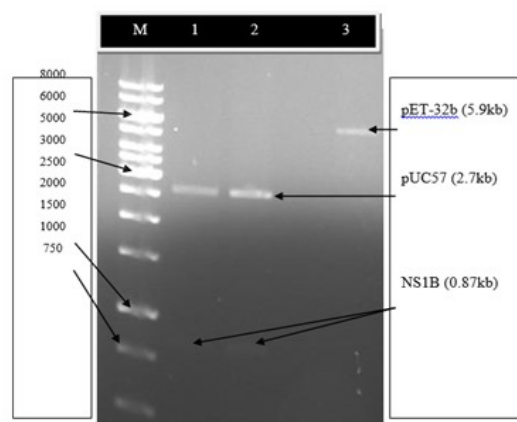


Figure 1: Restriction digestion by *HindIII*. M: 1 kb Marker, Lane 1, 2: pUC57 and NS1B gene, Lane 3: pET-32b.

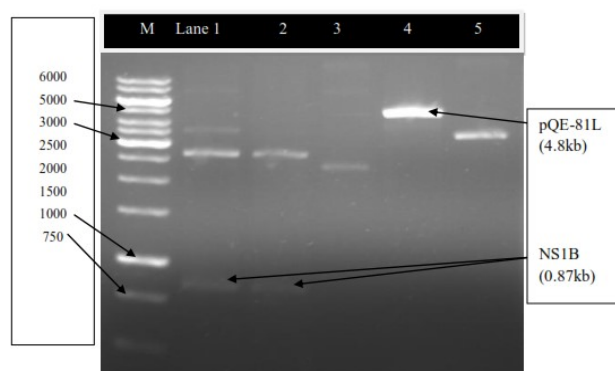


Figure 2: Plasmid isolation and digestion of pUC57-NS1B and pQE-81L M: 1kb Marker, Lane 1 and 2: pUC57-NS1B digested with *HindIII*, Lane 3: Miniprep of pUC57-NS1B, Lane 4: Double digestion of pQE-81L, Lane 5: Miniprep of pQE-81L.

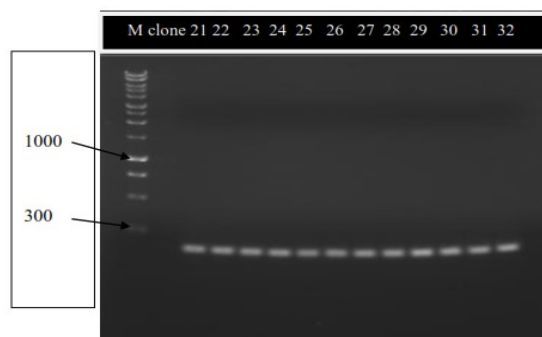


Figure 3: PCR of recombinant pQE-81L-NS1B.

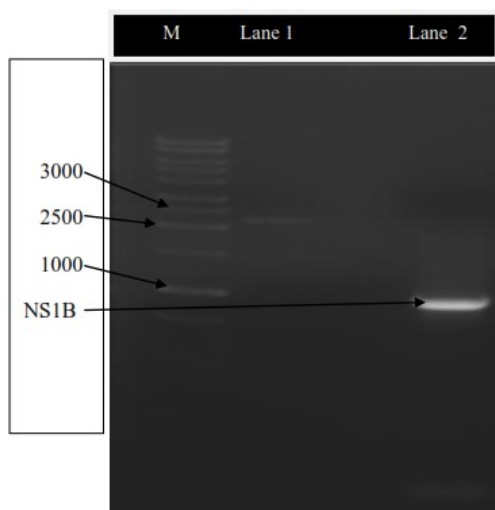


Figure 4: Miniprep of pUC57-NS1B and PCR amplification of NS1B. M: 1kb marker, Lane1: Miniprep of pUC57-NS1B, Lane 2: PCR amplification of NS1B gene.

The colonies were screened by PCR. All colonies screened gave negative result. As shown in Figure 6, the size of amplified band is smaller than 750 bp, indicating that only the pET-32a vector was transformed into the cells without the cloning of NS1B gene. The right band size to be expected is nearly 1500 bp (Figure 5).

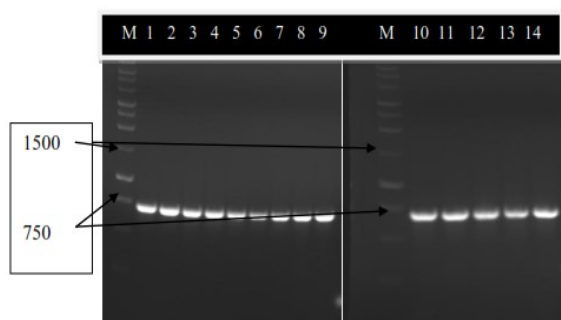


Figure 5: PCR of recombinant pET-32a-NS1B M: 1 kb Marker, Lane 1-14: Clone from 62-75.

## Discussion

Flu is a viral disease of global importance, it is caused by the influenza virus, which is an RNA virus diversity. NS1 protein persists only in the infected animal cells and not in vaccinated animals, so that, the vaccinated animals are not producing NS1 protein specific antibodies and this allows differentiation between vaccinated and natural infected animals (3). The function of NS1 protein during viral multiplication remains unclear, although a possible regulatory role in viral replication has been suggested from

studies of temperature sensitive mutant of NS1 (8). First, the NS1B protein may play an important role in counteracting the activity of antiviral gene products that are expressed in vero cells in an IFN-independent manner (9). NS1 also can suppression of host immune responses such as the apoptotic pathways and activation of phosphoinositide Kinase-3 (1).

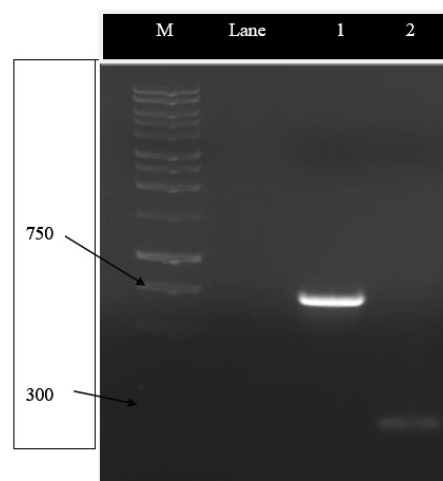


Figure 6: PCR amplification M: 1kb Marker, Lane 1: fragment without gene insert from pET-32aNS1B, Lane 2: fragment without gene insert from pQE-81L-NS1B.

NS1 gene was isolated from equine influenza virus A/equine 2/Suffolk /89(H3N8), and cloned into pGEX-3X vector then transformed in *E. coli* TG1 competent cell (10). pET-28 vector was employed to express the NS1 gene under the control of the T7 promoter and fused with His-Tag sequences (3). pQE-80L, the other type of vector with 6xHis-tag, was used to express NS1 protein of influenza A/Chicken/TH/KU14/04 (H5N1) (6). Other study showed that western blot assay for the rNS1 protein, can be applied in a DIVA strategy test and titer of NS1 antibody was low in bird's sera (11). Marzieh found that NS1 gene of influenza A H1N1 virus could be cloned and the rNS1 protein could be expressed using a bacterial protein translation system (12). It has been showed that multifunctional virulence factor heavily after isolation in comparison to FLUAV NS1protein (13). M2e-HA2 sequence of influenza A was cloned into pGS-21a vector to produce GST fusion protein (14).

Currently study, we have successful transformed pUC57-NS1B plasmid into *E. coli* BL21 (DE3). NS1B gene was successful isolated from the plasmid and cloned with PCR showed in figure 6. Designed primers used for PCR of NS1B showed successful amplification. First screening of pET-32b-NS1B colonies using white/blue method gave

negative result. Similar negative results were observed when screening colonies of pQE-81L-NS1B, and pET-32a-NS1B. Screening of colonies for pET-32a-NS1B showed a band of the size nearly 750bp, which is not as the expected band size of 1500bp. Screening of colonies for all vectors used showed only plasmids without any insertion of the NS1B gene. Cloning NS1B into pET-32b using single restriction digestion with HindIII, pQE-81L using double restriction digestion with (PstI and HindIII), pET-32a using double restriction digestion with (SacI and HindIII), gave unexpected result. This result may relate to re-ligation of digested vector for single digestion and uncompleted digestion for vectors of double restriction digestion.

A further construction of NS1 gene into pET32a, pET-32b, and pQE-81L vectors should be performed with best precaution of procedures to avoid any contamination and the use of appropriate restriction enzymes with high efficiency to get recombinant construction. The NS1B gene can also be cloned into other expression vectors according to the open reading frame and use other host.

Previous studies was isolated NS1 gene from equine influenza virus A/equine 2/Suffolk /89(H3N8), cloned into pGEX-3X vector and transformed in *E. coli* TG1 competent cell (10). NS1 gene from Influenza A (H9N2) was cloned and highly expressed using pET-28 vector (3). pQE-80L, the other type of vector with 6xHis-tag, was used to express NS1 protein of influenza A/Chicken/TH/KU14/04 (H5N1) (6). Very limited studies have isolated NS1B gene using vectors other than those used in this study.

This study was faced some limitations. First, several repetitions of digestion and ligation of enzyme are more fastidious. Second, difficult insertion of plasmids in competent host cells. Furthermore, choosing one appropriate vector is better than several vectors with single enzyme digestion. It is suggested that the overexpression of the constructed recombinant into expression system are being studied and work on purification can also be performed using immobilized affinity chromatography resin.

## Conclusion

According to the results of this study, it could be concluded that the NS1B gene was successful transformed, cloned and isolated from *E. coli* BL21 (DE3). Transformation, cloning and isolation of pET32a, pET-32b, pQE-81L were successful done in *E. coli*. This study was failed for ligation of NS1B gene in the expressing vectors.

## References

1. Marzieh S, Mojgan B, Fatemeh Y, Vahidreza Y, Elham T, Bahram K. Cloning and expression of influenza H1N1 NS1 protein in *Escherichia coli* BL21. Iran J Biotechnol. 2014; 12(1):12625: 1-5 DOI: 10.5812/IJB.12625.
2. Manasatiengkij W, Lekcharoensuk P, Upragarin N. Expression and purification of NS1 protein of highly pathogenic avian influenza virus H5N1 in *Escherichia coli*. Kasetsart J Nat Sci. 2008; 42:485-494.
3. Fang KW, Xio FY, Yi CW, Cun Z, Li HX, Si DL. Cloning, prokaryotic expression, and antigenicity analysis of H9N2 swine influenza virus. Sci Rep. 2016; 6:895-900.
4. Donelan N, Dauber B, Wang X, Basler C, Wolff T, Garcia SA. The N- and C-terminal domains of the NS1 protein of influenza B virus can independently inhibit IRF-3 and beta interferon promoter activation. J Virol. 2004; 78(21):11574-82, DOI: 10.1128/JVI.78.21.11574-11582.
5. Hatada E, Takezawa T, Fukuda R. Specific binding of influenza A virus NS1 protein to the virus minus-sense RNA in vitro. J Gen Virol. 1992; 73(1):17-25, DOI: 10.1099/0022-1317-73-1-17
6. Manasatiengkij W, Lekcharoensuk P, Upragarin N. Expression and purification of NS1 protein of highly pathogenic avian influenza virus H5N1 in *Escherichia coli*. Kasetsart J Nat Sci. 2008; 42:485-494.
7. Masoud M, Mehdi G, Hadi T, Reza G. Cloning, expression and purification of M2e-HA2 from Influenza A virus in *Escherichia coli*. J Vet Res. 2015; 19(2):124-129.
8. Binns M, Ely. Expression of the non-structural protein NS1 of influenza virus and detection of anti-NS1 antibody in serum. European Patent Application. 1996; 33, no: 96300681.2:1-20.
9. Dauber B, Heins G, Wolff T. The Influenza B virus nonstructural NS1 protein is essential for efficient viral growth and antagonizes beta interferon induction. J Virol. 2003; 78(4): 1865-1872, DOI: 10.1128/JVI.78.4.1865-1872.
10. Birch Ma I, Rowan A, Jane PJ, Mumford J, Binns M. Expression of the nonstructural protein NS1 of equine influenza A virus: detection of Anti-NS1 antibody in post infection equine sera. J Virol Methods. 1997; 65(2):255-263, DOI: 0166-0934.
11. Ramin S, Mehdi VM, Mohammad BHS, Farhid H. The Cloning of non-structural-1 (NS1) gene of H9N2 subtype of avian influenza virus in pGEX-4T-1 and pMAL-c2X plasmids and expression in *Escherichia coli* DH5a strain. Adv Biosci Biotechnol. 2012; 3:283-289, DOI:10.4236/abb.2012.33040.
12. Muhammad J, Muhammad HZ. Molecular cloning and expression of NS1 of the influenza "A" virus from different subtypes, master project, School of Life Sciences, Sodertorns University; 2011.
13. Patzina C, Botting CH, Garcia SA, Randall RE, Hale BG. Human interactome of the influenza B virus NS1 protein. J Gen Virol. 2017; 98:2267-2273, DOI:10.1099/jgv.0.000909.
14. Li ZF, Liu Y, Rui Y, Ke FS, Jin LY. Cloning and phylogenetic analysis of NS1 Gene of H3N2 subtype swine influenza virus from different isolates in chongqing china 2010-2011. J Anim Vet Adv. 2012; 11(12):2126-2128, DOI: 10.3923/javaa.2012.2126.2128.