Comparison of DNA extraction efficiency between two commercial kits on whole blood, swab, and plasma samples

Minh Quan Hoang[1], Huu An Nguyen[1], Bich Huong Bui[1], Thuy Quynh Nguyen[1], Thuy Ngan Nguyen[1], Thi Hien Nguyen[1], Thi Phuong Nguyen[1], Thi Hoi Le[1, 2], Tien Dung Pham[1*]

[1]Phacogen Institute of Technology, B4 Building, Pham Ngoc Thach Street, Kim Lien Ward, Hanoi, Vietnam
[2]Hanoi Medical University, 1 Ton That Tung Street, Kim Lien Ward, Hanoi, Vietnam

Received 13 December 2024; revised 17 December 2024; accepted 31 December 2024

Abstract:

Whole blood, swab, and plasma samples are widely used in molecular biology testing in Vietnam. However, most DNA extraction kits currently in use are imported and rely on silica column technology, which can be challenging to integrate into automated or high-throughput workflows. To address this limitation, PCG® MagBead DNA extraction kit (Phacogen, Vietnam) (PCG) was developed for DNA extraction from whole blood, swab, and plasma samples. Notably, the PCG kit is compatible with both manual and automated workflows across various automated systems. This study evaluated the performance of the PCG kit compared to the QIAamp DNA mini kit (Qiagen) (QIA) using 90 clinical samples, comprising 30 whole blood samples, 30 swab samples, and 30 plasma samples. DNA quality was assessed based on concentration, A260/280 purity, and real-time polymerase chain reaction (PCR) quantification of the beta-actin gene. Selected high-concentration samples were further analysed using agarose gel electrophoresis to evaluate DNA size and integrity. The results showed comparable DNA quality between the two kits, with no significant differences in concentration or purity. These findings indicate that the PCG kit performs equivalently to the QIA kit and is a cost-effective, reliable alternative for DNA extraction across diverse clinical samples in molecular biology applications.

Keywords: **DNA extraction, magnetic technology, Phacogen, plasma, swab, whole blood.**

Classification numbers: **3.5, 3.6**

1. Introduction

DNA extraction is a fundamental and routine technique in both research and diagnostic laboratories. Currently, most DNA extractions are performed using commercial kits. The success of molecular analyses, including quantitative PCR (qPCR), next-generation sequencing (NGS), and sanger sequencing (SS), depends heavily on the quality of the extracted DNA [1]. Extracting DNA from biological specimens such as blood, swabs, and plasma is critical for various medical and research applications, including genetic studies, disease diagnosis, and early detection. The quality of the extracted DNA significantly impacts the accuracy and reliability of analytical and diagnostic outcomes. Therefore, selecting an appropriate DNA extraction kit and technology is essential.

Despite their widespread use, current DNA extraction methods and commercial kits have limitations. First, some require the use of toxic and volatile chemicals like phenol, chloroform, and SDS [2]. Second, many involve complex and time-consuming procedures that are impractical for routine use [3]. Third, most commercial kits are designed for specific sample types, limiting their flexibility and compatibility across different specimen types [1]. The market offers a variety of DNA extraction kits employing diverse technologies and methods, each tailored to optimise DNA extraction from specific sample types. The QIAamp DNA mini kit (Qiagen) and the PCG® MagBead DNA extraction kit (Phacogen) are two widely used kits representing different technological approaches. The QIAamp kit employs silica column technology, while the PCG® MagBead kit uses magnetic separation technology. Both have unique advantages and limitations, and the choice between them often depends on the specific requirements of the application.

This study aims to compare the performance of these two DNA extraction kits using blood, swab, and plasma samples. Key parameters such as extraction efficiency, DNA purity, and compatibility with molecular biology techniques (e.g., real-time PCR) will be evaluated. By providing a detailed analysis of these factors, the study seeks to offer valuable insights into the efficiency and reliability of each kit, enabling researchers and clinicians to make informed choices about the most suitable DNA extraction tools for their specific needs.

*Corresponding author: Email: dungpt@phacogen.com

2. Materials and methods

2.1. Materials

Specimens, including whole blood, swabs, and plasma, were randomly collected at Phacolab Testing Centre (Vietnam), with a quantity of 30 samples each. The extraction chemicals included the PCG® MagBead DNA extraction kit (Phacogen), manufactured in an ISO 13485-certified facility and classified as an *in vitro* diagnostic (IVD) product, and the QIAamp DNA mini kit (Qiagen). Real-time PCR chemicals comprised HotStarTaq DNA polymerase (Qiagen), and primer and probe (IDT) specifically amplifying the *ACTB* gene segment encoding human beta-actin (Table 1). Quantitative chemicals for total DNA concentration in serum samples included the AccuClear® ultra high sensitivity dsDNA quantitation kit with DNA standards (Biotium). Electrophoresis chemicals included 10X TBE (Thermo Fisher), GelRed® nucleic acid gel stain (Biotium), and ready-to-use 1 kb DNA ladder (Biotium).

Table 1. Primer and probe sequences used in the study [4].

Order	Primer	Sequences (5'-3')	T_m (°C)
1	Beta-actin-F	ACCGAGCGCGGCTACAG	66.3°C
2	Beta-actin-R	CTTAATGTCACGCACGATTTCC	61.9°C
3	Beta-actin-P	TTCACCACCACGGCCGAGC	69°C

2.2. Methods

2.2.1. Study design

DNA was extracted from whole blood, swab, and plasma samples using the PCG® MagBead DNA extraction kit (PCG) and the QIAamp DNA mini kit (QIA). The quality of the extracted DNA was assessed based on the following indicators: DNA concentration and purity were determined using either the Nanodrop or Qubit 4.0, with the latter being used for plasma samples due to the limitations of Nanodrop in accurately measuring plasma DNA concentrations. The level of DNA degradation was evaluated through the smear pattern observed in electrophoresis, performed only on high-concentration DNA samples (>50 ng/µl). The suitability of the DNA for molecular biology analyses was further assessed via real-time PCR targeting the human beta-actin gene segment.

2.2.2. Real-time polymerase chain reaction detects the ACTB gene segment encoding human beta-actin

DNA samples extracted using both PCG® MagBead DNA extraction kit (Phacogen) and QIAamp DNA mini kit (Qiagen) were used as templates for 25 µl real-time PCR reactions containing 1X PCR Buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 U HotStarTaq DNA polymerase (Qiagen). In these reactions, 5 µl of extracted DNA was used as template and the reaction temperature cycle included: 95°C, 15 min; 40 cycles (95°C, 15 s; 58°C, 30 s (read signal at ROX channel); 72°C, 30 s).

2.2.3. Statistical analysis

Pairwise comparison statistical tests were conducted using the Kruskal-Wallis method to evaluate differences in DNA extraction efficiency between the two kits. Calculations and graphical representations were performed using R and RStudio software, ensuring rigorous and reproducible data analysis.

3. Results and discussion

3.1. Results of comparison of concentration and A260/280 purity of DNA samples after extraction of clinical samples using both kits

The results of comparing the concentration and purity of the DNA samples, determined at A260/280 wavelength after extraction, are shown in Tables 2-4.

Table 2. Concentration and purity results of DNA samples from whole blood samples.

Order	Sample ID	PCG® MagBead DNA extraction kit		QIAamp DNA mini kit	
		Concentration (ng/µl)	*A260/280*	*Concentration (ng/µl)*	*A260/280*
1	762311850	49.2	1.77	52.3	1.83
2	762311965	69.9	1.87	73.5	1.85
3	762311973	87.3	1.88	75.3	1.79
4	762311970	57.4	1.85	61.2	1.84
5	762311969	71.7	1.88	78.5	1.84
6	762311967	50.3	1.72	50.7	1.81
7	762311966	87.8	1.86	75.8	1.84
8	762312108	86.1	1.85	72.6	1.74
9	762312110	67.7	1.78	76.7	1.72
10	762310279	122.2	1.82	116	1.81
11	762312109	121.9	1.82	105.9	1.79
12	762312111	73.3	1.82	72.3	1.8
13	762312034	32.2	1.78	36.9	1.73
14	762312036	36.9	1.74	36	1.73
15	762312031	54.3	1.83	60.2	1.84
16	762312038	56.2	1.77	53.9	1.76
17	762312040	22.1	1.72	18.8	1.77
18	762312233	49.2	1.8	52.6	1.82
19	762312186	58.5	1.73	58.2	1.73
20	762312159	69.1	1.74	69.7	1.66
21	762312161	54.3	1.78	57.2	1.72
22	762312179	67	1.75	59	1.78
23	762312162	63.1	1.76	60.2	1.74
24	762312185	77.3	1.77	76.9	1.69
25	762312184	59.5	1.82	53.2	1.74
26	762312293	29.9	1.82	34.4	1.85
27	762312294	20.8	1.83	26	1.81
28	762312295	21.9	1.8	19.1	1.76
29	762312296	20.1	1.83	24.2	1.81
30	762312297	37.1	1.84	39.4	1.75

Table 3. Concentration and purity results of DNA samples from swab samples.

Order	Sample ID	PCG® MagBead DNA extraction kit		QIAamp DNA mini kit	
		Concentration (ng/µl)	*A260/280*	*Concentration (ng/µl)*	*A260/280*
1	78231738	3	1.84	3.5	1.91
2	78231214	7.4	1.86	9.1	2.02
3	78231776	40.1	1.86	45.7	1.79
4	78231458	6	1.97	4.8	2.07
5	78231467	28.3	1.85	44.7	1.85
6	78231739	156.3	1.97	134.7	1.93
7	78231455	9.5	1.89	10.6	1.74
8	78231456	7.5	1.73	9	1.8
9	78231453	20.5	1.74	20.3	1.81
10	78231211	81.8	1.81	95.4	1.83
11	78231213	123	1.82	95.5	1.79
12	78231215	12.4	1.86	6.3	1.75
13	78231212	38.1	1.79	36.2	1.81
14	78231731	9.9	1.71	14.1	1.65
15	78231737	10.8	1.75	12.9	1.79
16	78231466	5.1	1.97	3.4	1.94
17	78231373	74.8	1.83	79.9	1.84
18	78231094	74.6	1.87	54	1.96
19	78231554	8.8	1.92	10.8	2.25
20	78231533	17.7	1.81	20.2	1.98
21	78230661	87.5	1.85	88.7	1.85
22	78231093	11.4	1.91	5.9	2.02
23	78231096	8.6	1.8	9.2	1.93
24	78231372	22.1	1.9	24.6	1.89
25	78231092	165.1	1.86	143.8	1.84
26	78231842	73.4	1.85	56.6	1.83
27	78231091	109.3	1.8	104.4	1.83
28	78231826	17.3	1.79	17.5	1.85
29	78231535	36.1	1.78	43.2	1.76
30	78231553	3.9	1.88	3.9	2.1

Table 4. Concentration of DNA samples from plasma samples.

Order	Sample ID	PCG® MagBead DNA extraction kit	QIAamp DNA mini kit
		Concentration (ng/µl)	*Concentration (ng/µl)*
1	732312486	1.06	0.768
2	762312291	1.92	2.57
3	732312666	1.82	1.55
4	762311967	0.952	0.89
5	762312292	2.1	1.37
6	762311969	1.93	1.63
7	762312079	1.27	1.47
8	762311970	2.12	2.8
9	762311973	1.04	1.04
10	732312485	3.19	3.56
11	762311965	1.91	1.55
12	762312111	1.76	1.53
13	762312034	1.96	1.08
14	762312036	1.46	1.68
15	762312031	1.9	1.83
16	762312038	2.65	1.42
17	762312040	1.34	1.34
18	762312233	1.68	1.15
19	762312186	1.28	0.872
20	762312159	1.96	1.92
21	762312161	1.26	1.85
22	762312179	0.932	1.15
23	762312162	0.648	0.688
24	762312185	1.62	1.54
25	762312184	2.1	2.83
26	762312293	1.12	1.16
27	762312294	1.4	1.46
28	762312295	3.14	3.53
29	762312296	1.54	1.17
30	762312297	3.04	2.36

Based on the DNA concentration and purity measurements using Nanodrop and Qubit (for plasma samples), a t-test was conducted to compare the two DNA extraction methods (Fig. 1). For whole blood samples, the median DNA concentration was 57.95 ng/µl for the PCG kit and 58.6 ng/µl for the Qiagen kit. Similar results were observed for swab samples, with median concentrations of 19.10 ng/µl (PCG kit) and 20.25 ng/µl (Qiagen kit), and for plasma samples, with 1.72 ng/µl (PCG kit) and 1.50 ng/µl (Qiagen kit). The median purity values (A260/280) were also comparable between the two kits. For whole blood samples, the PCG kit yielded a median purity of 1.810, while the Qiagen kit yielded 1.785. Similarly, for swab samples, the median purity values were 1.850 (PCG kit) and 1.840 (Qiagen kit). Statistical analysis indicated no significant difference in DNA concentration or purity between the two kits (p-value > 0.05). These results suggest that the DNA quality obtained using the PCG® MagBead DNA extraction kit (Phacogen) is equivalent to that obtained using the QIAamp DNA mini kit (Qiagen). Further supporting these findings, other studies have demonstrated the advantages of magnetic bead-based DNA extraction over silica column-based methods. For instance, magnetic bead technology has shown superior efficiency in

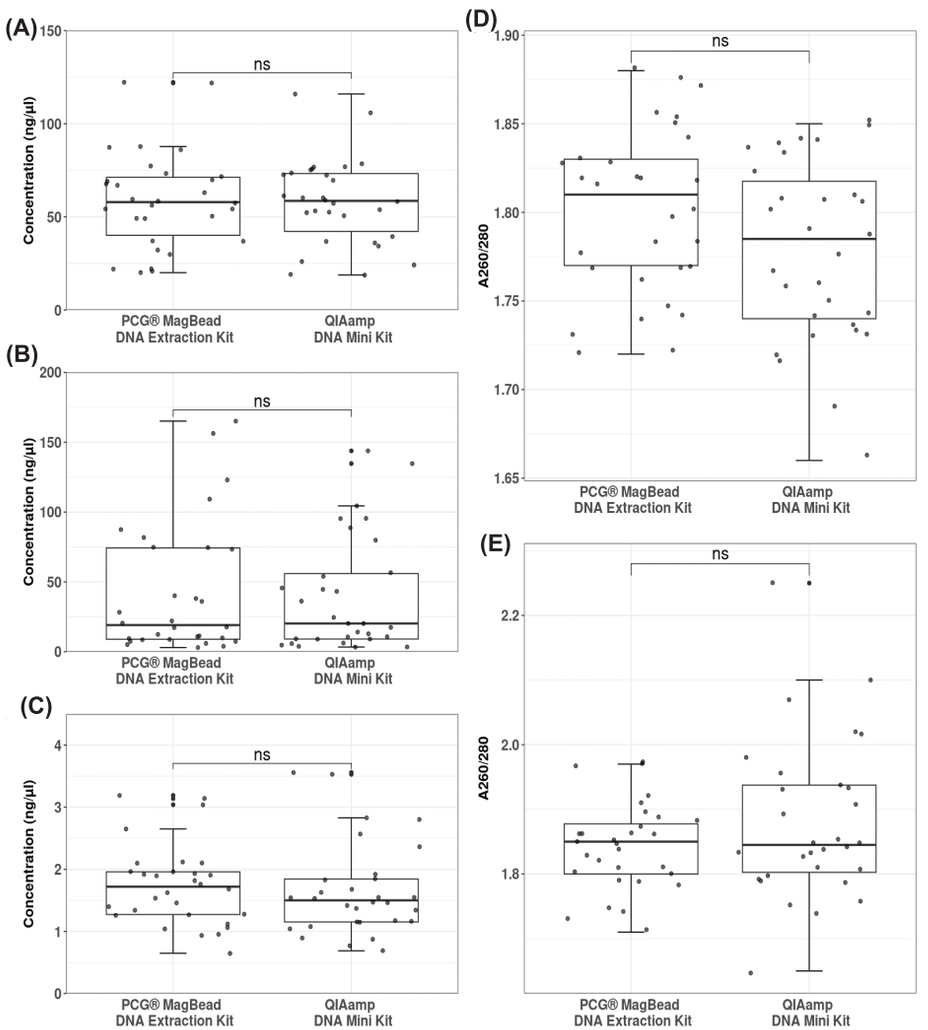

Fig. 1. Results of comparing the concentration and purity of DNA samples after extraction by two methods: (A) DNA concentration comparison in blood samples; **(B)** DNA concentration comparison in swab samples; **(C)** DNA concentration comparison in plasma samples; **(D)** DNA purity comparison in blood samples; **(E)** DNA purity comparison in swab samples, ns. not statistically significant.

3.2. Results of comparing the size of DNA samples after extracting clinical samples using both kits

In this study, some DNA samples (both high and low concentration) from whole blood and swab samples were electrophoresed to compare the quality between the two kits (Fig. 2). Plasma samples were not used for electrophoresis due to their very low DNA concentration of approximately 1 ng/µl.

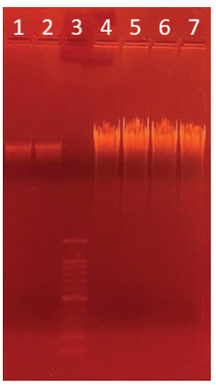

Fig. 2. Electrophoresis results of DNA extracted from swab samples and whole blood samples. Track 1: Swab DNA sample 78231453 extracted by PCG; Track 2: Swab DNA sample 78231453 extracted by QIA; Track 3: Standard DNA ladder; Track 4: Whole blood DNA sample 762310279 extracted by PCG; Track 5: Whole blood DNA sample 762310279 extracted by QIA; Track 6: Whole blood DNA sample 762312109 extracted by PCG; Track 7: Whole blood DNA sample 762312109 extracted by QIA.

extracting DNA from challenging samples, such as skeletal remains, which require high-efficiency recovery methods. This was highlighted in the study by I.M. Cândido, et al. (2014) [5]. Additionally, magnetic bead technology has proven beneficial in applications requiring high-quality DNA, such as tumour-related genetic material extraction in cancer research. F. Zamuner, et al. (2024) [6] demonstrated that automated extraction using magnetic bead-based kits improved throughput without compromising the efficiency of target gene amplification in PCR systems. These findings emphasise the reliability and versatility of magnetic bead-based DNA extraction technologies for various molecular biology applications.

The results showed that DNA samples extracted from swab and whole blood samples using both methods were similar in terms of integrity and purity, as indicated by comparable smear patterns observed in electrophoresis. For DNA sample 78231453 (lanes 1 and 2), a moderately bright DNA band was observed, corresponding to a moderate DNA concentration (~20 ng/µl) and moderate fragmentation. Similarly, for DNA samples 762310279 and 762312109 (lanes 4-7), a bright DNA band was detected, indicative of a very high DNA concentration (~110 ng/µl). These findings demonstrate that both extraction methods produce DNA of comparable quality, with sufficient integrity and purity for downstream applications requiring intact or minimally fragmented DNA.

3.3. Comparison of the real-time polymerase chain reaction analysis ability of DNA samples after extraction of clinical samples using both kits

The research team evaluated the ability to analyse the quality of extracted DNA samples using real-time PCR technology on the human beta-actin gene. Since the patient samples all contained human gene markers, and this gene is one of the housekeeping genes expressed in most human tissues. The results of real-time PCR of the human *ACTB* gene on DNA samples extracted by the two kits are shown in Tables 5-7.

Table 5. Real-time polymerase chain reaction results of beta-actin gene on DNA samples extracted from whole blood samples.

Order	Sample ID	C_T_PCG	C_T_QIA
1	762311850	37.12	42.67
2	762311965	40.755	36.857
3	762311973	38.725	38.338
4	762311970	38.252	39.603
5	762311969	38.561	39.861
6	762311967	35.773	39.045
7	762311966	36.13	35.012
8	762312108	31.633	33.516
9	762312110	36.687	34.693
10	762310279	38.608	39.863
11	762312109	41.076	39.583
12	762312111	35.122	36.294
13	762312034	30.515	31.651
14	762312036	33.524	38.861
15	762312031	35.699	36.603
16	762312038	38.284	38.074
17	762312040	34.769	36.856
18	762312233	31.297	33.007
19	762312186	36.673	36.698
20	762312159	37.486	39.365
21	762312161	40.127	44.881
22	762312179	38.586	38.803
23	762312162	37.805	40.008
24	762312185	36.4	37.614
25	762312184	40.575	40.229
26	762312293	39.342	40.807
27	762312294	40.288	40.357
28	762312295	42.47	42.756
29	762312296	40.695	40.298
30	762312297	38.632	37.784

Table 6. Real-time polymerase chain reaction results of beta-actin gene on DNA samples extracted from swab samples.

Order	Sample ID	C_T_PCG	C_T_QIA
1	78231738	29.937	31.525
2	78231214	31.425	32.296
3	78231776	35.561	36.125
4	78231458	35.246	35.588
5	78231467	33.284	36.905
6	78231739	37.81	38.251
7	78231455	29.895	30.406
8	78231456	30.114	39.449
9	78231453	38.167	33.837
10	78231211	35.626	35.61
11	78231213	33.699	34.945
12	78231215	34.426	38.076
13	78231212	35.874	36.6
14	78231731	32.547	33.592
15	78231737	31.298	31.2
16	78231466	31.598	31.988
17	78231373	30.36	34.432
18	78231094	32.196	34.804
19	78231554	31.648	31.617
20	78231533	32.146	33.595
21	78230661	32.065	34.038
22	78231093	35.537	40.524
23	78231096	29.254	32.418
24	78231372	33.514	33.595
25	78231092	33.687	35.39
26	78231842	27.894	28.313
27	78231091	29.923	31.193
28	78231826	33.869	36.439
29	78231535	41.298	43
30	78231553	26.788	28.293

Table 7. Real-time polymerase chain reaction results of beta-actin gene on DNA samples extracted from plasma samples.

Order	Sample ID	C_T_PCG	C_T_QIA
1	732312486	31.451	34.119
2	762312291	35.217	35.854
3	732312666	38.032	37.135
4	762311967	34.557	36.49
5	762312292	33.978	34.571
6	762311969	35.593	36.567
7	762312079	34.56	37.987
8	762311970	37.336	39.08
9	762311973	37.788	39.068
10	732312485	38.412	40.286
11	762311965	39.153	39.795
12	762312111	33.362	35.645
13	762312034	40.41	41.793
14	762312036	38.06	40.805
15	762312031	39.403	41.378
16	762312038	40.21	41.383
17	762312040	32.825	35.53
18	762312233	37.605	36.399
19	762312186	37.601	36.845
20	762312159	38.236	39.699
21	762312161	41.026	40.904
22	762312179	40.537	39.384
23	762312162	34.704	36.489
24	762312185	37.052	40.155
25	762312184	34.891	35.071
26	762312293	36.549	37.434
27	762312294	37.266	38.649
28	762312295	33.301	32.188
29	762312296	35.875	33.625
30	762312297	35.941	33.733

The research team performed a t-test to compare the real-time PCR results between the two methods (Fig. 3). It was found that the cycle threshold (CT) value when amplifying the *ACTB* gene in DNA samples extracted by the PCG kit (38.03) was earlier than in those extracted by the QIA kit (38.83). The CT value in DNA samples extracted from swabs or plasma by the PCG kit was also earlier than in those extracted by the QIA kit. However, the statistics showed no statistically significant difference. Thus, it is possible to preliminarily evaluate that the quality of DNA samples extracted from blood, swabs, and plasma by the PCG and QIA kits is similar, and both are suitable for real-time PCR tests.

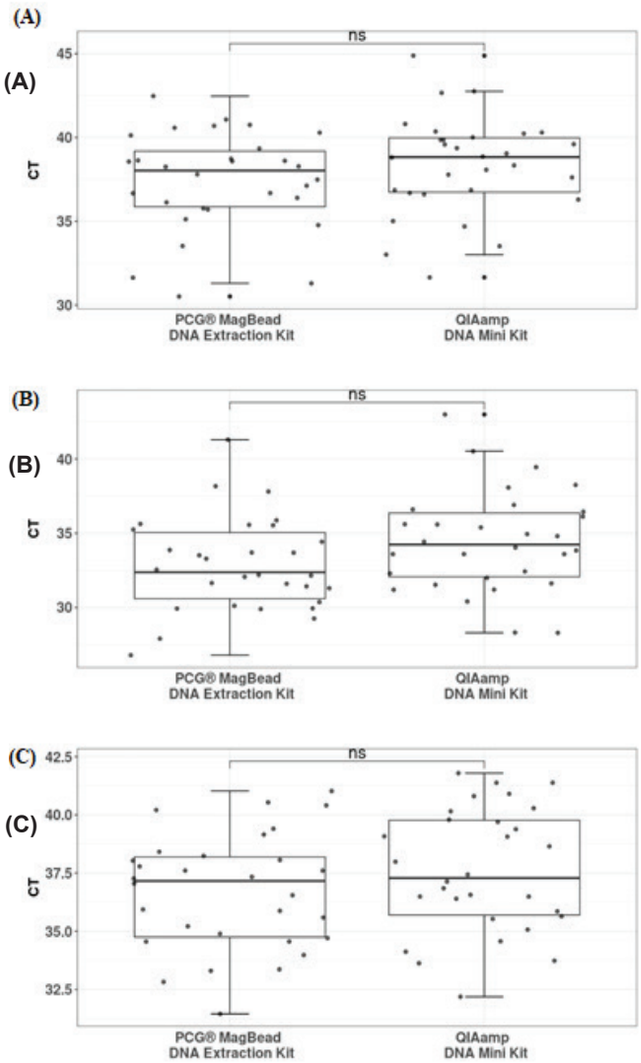

Fig. 3. Statistical comparison of real-time polymerase chain reaction threshold cycles of *ACTB* gene in DNA samples extracted by two kits. (A) Blood samples; **(B)** Swab samples; **(C)** Plasma samples. CT: cycle threshold; ns: not statistically significant.

High DNA concentration or favourable A260/280 purity ratios alone do not guarantee DNA quality that meets the standards required for molecular biology techniques such as real-time PCR. Real-time PCR is highly sensitive and can be affected by inhibitors present in DNA samples, such as polysaccharides, phenols, guanidine isothiocyanates, and humic acids, which have absorption wavelengths similar to nucleic acids [7-10]. These contaminants may

not be detectable through spectrophotometric methods like Nanodrop, potentially leading to false-negative results or significant differences in CT values, as reported in previous studies [11-15]. To address this issue, the inclusion of housekeeping genes can help identify false-negative results. The *ACTB* gene, which encodes the highly conserved cytoskeletal protein beta-actin, is commonly used as an internal positive control to detect PCR inhibitors in diagnostic samples [16]. This approach ensures more reliable results by confirming the absence of inhibitors that could interfere with PCR amplification.

4. Conclusions

The research team conducted a comparative study of the PCG® MagBead DNA extraction kit (Phacogen) and the QIAamp DNA mini kit (Qiagen) to evaluate DNA extraction efficiency. A total of 90 samples, including 30 blood samples, 30 swab samples, and 30 plasma samples, were analysed for DNA quality using Nanodrop, Qubit, agarose gel electrophoresis, and real-time PCR targeting the beta-actin gene. The findings revealed a high level of similarity in DNA quality between the two kits. Consistent with global studies, DNA extraction kits based on magnetic bead technology demonstrated significant advantages over silica column-based kits. These advantages, coupled with the results of this study, suggest that the PCG® MagBead DNA extraction kit, developed and manufactured in Vietnam, represents a cost-effective and efficient alternative for DNA extraction. This kit has the potential to enhance DNA recovery and reduce costs for researchers and molecular biology applications when widely adopted.

CRediT author statement

Minh Quan Hoang, Bich Huong Bui, Thuy Quynh Nguyen, Thuy Ngan Nguyen, Thi Hien Nguyen, Thi Phuong Nguyen: Writing; Huu An Nguyen: Conceptualisation, Writing - Reviewing and Editing; Thi Hoi Le, Tien Dung Pham: Conceptualisation.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] S. Zielińska, P. Radkowski, A. Blendowska, et al. (2017), "The choice of the DNA extraction method may influence the outcome of the soil microbial community structure analysis", *MicrobiologyOpen*, **6(4)**, DOI: 10.1002/mbo3.453.

[2] Y. Xia, F. Chen, Y. Du, et al. (2019), "A modified SDS-based DNA extraction method from raw soybean", *Bioscience Reports*, **39(2)**, DOI: 10.1042/BSR20182271.

[3] S. Sharma, M. Riti, S. Kumar, et al. (2019), "A simple and cost-effective freeze-thaw based method for plasmodium DNA extraction from dried blood spot", *Iranian Journal of Parasitology*, **14(1)**, pp.29-40.

[4] M.M.A. Qattan, M.K. Shier, M.M.A. Alwahed, et al. (2013), "Salamander-derived, human-optimized nag protein suppresses collagen synthesis and increases collagen degradation in primary human fibroblasts", *BioMed Research International*, DOI: 10.1155/2013/384091.

[5] I.M. Candido, L.S.D.A. Bezerra, M.F.D. Mota, et al. (2014), "DNA extraction from human bone: Comparison of magnetic bead and silica column techniques", *Revista Odontológica do Brasil Central*, **23(66)**, DOI: 10.36065/robrac.v23i66.887.

[6] F. Zamuner, A.R. Lopez, A.G. Negron, et al. (2024), "Evaluation of silica spin-column and bead formats for rapid promoter DNA methylation analysis by qMSP in clinical and point-of-care settings", *Cancer Research*, **84**, DOI: 10.1158/1538-7445.AM2024-7288.

[7] R. Boom, C.J. Sol, M.M. Salimans, et al. (1990), "Rapid and simple method for purification of nucleic acids", *Journal of Clinical Microbiology*, **28(3)**, pp.495-503, DOI: 10.1128/jcm.28.3.495-503.1990.

[8] J. Sambrook, D.W. Russell (1989), "*In vitro* amplification of DNA by the polymerase chain reaction", *Molecular Cloning*, Cold Spring Harbor Laboratory Press, pp.6-36.

[9] C. Schrader, A. Schielke, L. Ellerbroek, et al. (2012), "PCR inhibitors-occurrence, properties and removal", *Journal of Applied Microbiology*, **113(5)**, pp.1014-1026, DOI: 10.1111/j.1365-2672.2012.05384.x.

[10] I.G. Wilson (1997), "Inhibition and facilitation of nucleic acid amplification", *Applied and Environmental Microbiology*, **63(10)**, pp.3741-3751, DOI: 10.1128/aem.63.10.3741-3751.1997.

[11] A. Das, E. Spackman, M.J.P. Jackwood, et al. (2009), "Removal of real-time reverse transcription polymerase chain reaction (RT-PCR) inhibitors associated with cloacal swab samples and tissues for improved diagnosis of Avian influenza virus by RT-PCR", *Journal of Veterinary Diagnostic Investigation*, **21(6)**, pp.771-778, DOI: 10.1177/1040638709021006.

[12] A. Das, E. Spackman, D. Senne, et al. (2006), "Development of an internal positive control for rapid diagnosis of avian influenza virus infections by real-time reverse transcription-PCR with lyophilized reagents", *Journal of Clinical Microbiology*, **44(9)**, pp.3065-3073, DOI: 10.1128/jcm.00639-06.

[13] J. Hedman, A. Nordgaard, C. Dufva, et al. (2010), "Synergy between DNA polymerases increases polymerase chain reaction inhibitor tolerance in forensic DNA analysis", *Analytical Biochemistry*, **405(2)**, pp.192-200, DOI: 10.1016/j.ab.2010.06.028.

[14] B. Hoffmann, M. Beer, S.M. Reid, et al. (2009), "A review of RT-PCR technologies used in veterinary virology and disease control: Sensitive and specific diagnosis of five livestock diseases notifiable to the World Organisation for Animal Health", *Veterinary Microbiology*, **139(1-2)**, DOI: 10.1016/j.vetmic.2009.04.034.

[15] P. Rådström, R. Knutsson, P. Wolffs, et al. (2004), "Pre-PCR processing: Strategies to generate PCR-compatible samples", *Molecular Biotechnology*, **26**, pp.133-146, DOI: 10.1385/MB:26:2:133.

[16] A. Das, M.Y. Deng, S. Babiuk, et al. (2017), "Modification of two capripoxvirus quantitative real-time PCR assays to improve diagnostic sensitivity and include beta-actin as an internal positive control", *Journal of Veterinary Diagnostic Investigation*, **29(3)**, pp.351-356, DOI: 10.1177/104063871769560.