

Extraction Method Comparison for The Detection of Porcine DNA in Cosmetic Products

Fadilah Mohd Hassan*, Siti Aminah Ibrahim, Ummu Nabihah Nordin

Malaysia Halal Analisis Centre, Jabatan Kemajuan Islam Malaysia, Bandar Baru Enstek, Negeri Sembilan, Malaysia.

*Corresponding author: Fadilah Mohd Hassan, Malaysia Halal Analisis Centre, Jabatan Kemajuan Islam Malaysia, Bandar Baru Enstek, Negeri Sembilan, Malaysia.; fadilah_mhassan@islam.gov.my

Abstract: Natural ingredients in cosmetic products, such as glycerine and collagen, may be derived from pigs, which is prohibited in Islam. The purpose of this study is to discover the most effective extraction methods for detection of porcine DNA in cosmetic products specifically lipstick, serum and cream moisturisers. Five different concentrations of porcine DNA were added to all these products. All three products were extracted using two different extraction methods and then subjected to a NanoPhotometer to determine the quality of the extracted DNA. The presence of porcine DNA in the samples was then amplified and determined by real-time PCR. For lipstick, no porcine DNA was detected in any samples using either the DNeasy® Mericon Food Kit or CTAB Wizard extraction methods. In contrast, for serum, all spiked porcine DNA was detected using the CTAB Wizard method but was undetected by the modified CTAB extraction method. Meanwhile, for cream moisturiser, both the DNeasy® Mericon Food Kit and PowerPrep™ Gelatin DNA Extraction Kit extraction methods successfully detected the presence of porcine DNA in all spiked samples. Different types of cosmetic products showed varying suitability for DNA extraction methods.

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1. Introduction

Cosmetics have been part of human life as early as 5000 BC, becoming part of Egyptian history. Nowadays, cosmetics have evolved and become an essential part of human daily life, serving not only practical and aesthetic purposes, but also reflecting social routines and lifestyle. Over time, cosmetics had dramatically changed including the ingredients, which may have been extracted naturally or produced synthetically. Therefore, many countries set requirements to ensure the cosmetics safety.

According to U.S Food, Drug, and Cosmetic Act (FD&C Act), under Section 201(i) and Malaysian Standard MS 2634:2019, cosmetic is perceived as substances intended to be used on external human body parts with purposes of enhancing, maintaining appearance and/or protecting the body. These regulations provide a clear difference between cosmetics from drugs and other products, thereby providing fundamental guidelines for assessing effectiveness, safety and impact on consumers. In contrast, the source of ingredients and entire manufacturing process, serve as main factor to differentiate between general cosmetics and *halal* cosmetics (Isa *et al.*, 2023).

Malaysian Standard MS 2634:2019 defines *halal* cosmetics as products that are permissible under Islamic law in which the ingredients are required to be free from harmful substances, *najs* (impurities) and derived from prohibited animals, in particular, pigs. Additionally, the substances are only acceptable if the animals are slaughtered in accordance with Shariah guidelines. The equipment or facilities are restricted to have any contamination from *najs* or materials from prohibited animals.

Despite strict regulatory requirements, the *halal* cosmetic sector has successfully penetrated the global market, gained recognition and continued to attract companies' worldwide (Isa *et al.*, 2023). State of the Global Islamic Economy (SGIE) Report 2024/2025 concluded that *halal* economy is currently developing rapidly which covers cosmetics, food and pharmaceuticals. Specifically, the estimated increase of USD 31 billion is anticipated between 2023 and 2028 in *halal* cosmetic sector. The largest spending market for *halal* cosmetics has already been established in Turkiye and Iran, whereas Indonesia, Bangladesh and Egypt are exhibiting rapid expansion which is mostly influenced by the digital development of the local cosmetic brands. Given that its demand continues to escalate within Asia, *halal* cosmetic is foreseen to transform into one of major *halal* economy. Europe and North America are experiencing rising demand in mainstream markets (Aoun & Tournois, 2015).

Consumer awareness on authenticity has significantly increased following the *halal* cosmetics development which any slight contamination may lead to *halal* integrity. Accordingly, Halal Cosmetic Standard (MS 2200:2008) is provided as a framework for *halal* certification of cosmetics. Ingredients derived from pigs are prohibited in *halal* cosmetics due to their non-compliance with Islamic law (Sugibayashi *et al.*, 2019). Thereby resulting in requirement to analyse cosmetic products for the presence of porcine-derived components to ensure the authenticity and integrity of *halal*-certified formulations. Glycerine, collagen, and fatty acids are widely used in cosmetic products and may be derived from various

sources, including porcine sources.(Sugibayashi *et al.*, 2019; Zabidi *et al.*, 2020). Due to its low cost and immense production, lard substituents have been widely used to increase the viscosity of some cosmetic products (Rohman & Man, 2011). Since all parts of pigs are prohibited from being eaten, used or touched in Islam, it is crucial to ensure the cosmetics ingredients used are devoid from any pig traces.

Multiple analytical methods have been established to meet this need which including of real-time polymerase chain reaction (RT-PCR or qPCR), enzyme-linked immunosorbent assay (ELISA) and Fourier-transform infrared spectroscopy (FTIR)(Kim *et al.*, 2018; Rohman & Man, 2011; S Abd-Gani *et al.*, 2019; Sari *et al.*, 2025; Zabidi *et al.*, 2020). This study utilized Polymerase Chain Reaction (PCR), a DNA-based approach that can amplify a low amount of porcine DNA and give a specific, sensitive, and fast result (S Abd-Gani *et al.*, 2019).

The qPCR method involves three main steps which are DNA denaturation, primer annealing, and strand extension by DNA polymerase. It used fluorescence probe to signal the amount of DNA in the samples, producing a quantitative, more sensitive, and rapid and more accurate result (Kim *et al.*, 2018). The study also stated that qPCR is an effective technique for identifying porcine-derived ingredients in cosmetic products. This method also allows real-time monitoring of target sequence amplification due to the fluorescence signal of the probe.

However, in these DNA-based analytical techniques, obtaining enough high-quality DNA from ingredients such as glycerine, collagen, and fatty acids used in cosmetic formulations presents significant challenges. This difficulty arises from the expected degradation of the limited DNA content, which is often exposed to harsh processing conditions such as elevated temperatures, strong pH treatments, and mechanical shear—during cosmetic manufacturing.

DNeasy® Mericon Food Kit, PowerPrep™ Gelatin DNA Extraction Kit, CTAB (Cetyltrimethylammonium Bromide) Wizard and modified CTAB method were chosen in this study due to their availability in Malaysia Halal Analysis Centre (MyHAC). DNeasy® Mericon Food Kit and PowerPrep™ Gelatin DNA Extraction Kit were commercial extraction kits, whereas the CTAB Wizard and modified CTAB methods were developed in-house.

2. Materials and Methods

2.1. Sample Preparation

Cosmetic samples, which are lipstick, serum and cream moisturiser were spiked with different concentration of lard DNA, as shown in Table 1. These spiked samples were

provided by the Department of Chemistry Malaysia and the concentration remained unknown throughout analysis to avoid any bias.

Table 1. Concentrations of Spiked Lard DNA (%) in Cosmetic Samples

Cosmetic Sample	Concentration of Spiked Lard DNA (%)				
	A	B	C	D	E
Lipstick	0.05	0	5	0.01	2
Serum	1	0.1	5	2	0
Cream Moisturiser	1	2	5	0	0.5

2.2. DNA Extraction

Each cosmetic sample type was subsampled, homogenized, and weighed in accordance with the requirements of the respective extraction method. Each cosmetic sample was then extracted using two different extraction techniques, as summarized in Table 2. The choice of extraction method was determined by the physical characteristics of the sample matrix. All samples were extracted in triplicate, alongside an extraction blank and an extraction control.

Table 2. Extraction Methods used for each Sample Type of Cosmetic Products

Cosmetic Sample	Extraction Method	
Lipstick	CTAB Wizard	DNeasy® Mericon Food Kit
Serum	CTAB Wizard	Modified CTAB
Cream Moisturiser	DNeasy® Mericon Food Kit	PowerPrep™ Gelatin DNA Extraction

2.3. Quantification of DNA Quality

The quality of extracted DNA such as concentration and purity was determined using a spectrophotometer (Implen NanoPhotometer NP80). DNA concentration was quantified by measuring absorbance at 260 nm, whereas purity was assessed using the 260/280 nm absorbance ratio. Prior to analysis, the elution buffer corresponding to each extraction method was employed as a blank to ensure accurate measurements.

2.4. Primer and Probe

The PCR primers and probe used for porcine DNA amplification were designed based on the method described by as shown in Table 3. The primers and probe were developed to target the porcine mitochondrial DNA especially on cytochrome *b* region (Tanabe *et.al.*, 2007). Primer specificity was evaluated during the in-house validation of the DNA porcine detection method.

Table 3. Sequence of Primers and Probe

Nucleotides	Sequences
Forward Primer	5'-CTTGCAAATCCTAACAGGCCTG-3'
Reverse Primer	5'-CGTTTGCATGTAGATAGCGAATAAC-3'
Probe	5'-(FAM)-ACAGCTTTCTCATCAGTTAC-(NFQ)(MGB)-3'

2.5. Real-time Polymerase Chain Reaction (qPCR)

The extracted DNA was subjected to qPCR amplification along with controls. A no-template control (NTC) was included as the negative control, while Certified Reference DNA of the target species served as the positive control. Each qPCR reaction was prepared in a total volume of 25 μ L, consisting of 2.5 μ L DNA template, 12.5 μ L TaqMan mastermix, 0.75 μ L primers, 0.50 μ L of probe, 2.5 μ L internal positive control (IPC), and 5.50 μ L sterile distilled water.

The qPCR reactions were performed on an Applied Biosystems Step One Plus under the cycling conditions summarized in Table 4.

Table 4. qPCR Cycling Conditions

Step	Temperature	Time	Cycles
Initial incubation	50 °C	2 min	1
Initial denaturation	95 °C	10 min	1
Denaturation	95 °C	15 s	45
Annealing/Extension	60 °C	1 min	45

3. Results

3.1. DNA Quality of Extracted DNA

The DNA quality of different DNA extraction methods was evaluated in three cosmetic matrices which are lipstick, serum, and cream moisturiser that spiked with varying concentrations of lard DNA. DNA concentration and purity were determined by spectrophotometer.

Based on Table 5, in lipstick samples, both the CTAB Wizard and the DNeasy® Mericon Food Kit yielded detectable amounts of DNA concentration for all spiking levels. The CTAB Wizard method produced relatively higher concentrations between 9.76–15.75 ng/ μ L, with acceptable purity values of 1.88–2.06. In contrast, the DNeasy® Mericon Food Kit yielded lower DNA concentrations (0.85–9.50 ng/ μ L) but demonstrated acceptable purity (1.62–2.17).

Table 5. Average of DNA Concentration and Purity based on Extraction Methods

Lipstick				
Concentration of spiked lard DNA (%)	Extraction Method			
	CTAB Wizard		DNeasy® Mericon Food Kit	
	DNA Concentration (ng/μl)	DNA Purity (A ₂₆₀ /A ₂₈₀)	DNA Concentration (ng/μl)	DNA Purity (A ₂₆₀ /A ₂₈₀)
0	15.12	1.97	0.92	2.17
0.01	13.29	1.88	2.27	1.62
0.05	9.76	2.06	9.50	1.91
2	15.75	1.90	2.07	1.65
5	12.70	1.90	0.85	2.05
Serum				
	CTAB Wizard		Modified CTAB	
	DNA Concentration (ng/μl)	DNA Purity (A ₂₆₀ /A ₂₈₀)	DNA Concentration (ng/μl)	DNA Purity (A ₂₆₀ /A ₂₈₀)
0	13.08	1.90	1.44	2.77
0.1	21.54	3.1	3.93	4.8
1	14.25	2.09	37.21	2.27
2	14.64	1.82	2.35	0.94
5	11.95	2.07	6.18	2.18
Cream Moisturiser				
	DNeasy® Mericon Food Kit		PowerPrep™ Gelatin DNA Extraction Kit	
	DNA Concentration (ng/μl)	DNA Purity (A ₂₆₀ /A ₂₈₀)	DNA Concentration (ng/μl)	DNA Purity (A ₂₆₀ /A ₂₈₀)
0	2.84	1.22	9.84	4.13
0.5	3.58	1.25	1.51	2.17
1	2.32	1.83	5.27	-15.78
2	1.88	1.16	2.89	-4.16
5	2.14	1.14	4.27	1.15

For serum, the CTAB Wizard method produced moderate DNA concentration of 11.95–21.54 ng/μl with purity values scores within the range of 1.82–3.1. The modified CTAB method showed more variable performance, with DNA concentrations ranging from 1.44 to 37.21 ng/μl and purity ratios fluctuating between 0.94–4.80 ratios. Remarkably, the modified CTAB method yielded substantially higher DNA concentration at 1% spiking which is 37.21 ng/μl.

In cream moisturiser, the DNeasy® Mericon Food Kit consistently produced low DNA yields between 1.88–3.58 ng/μl with poor purity ratios of 1.14–1.83. Conversely, the PowerPrep™

Gelatin DNA Extraction Kit yielded higher concentrations of 1.51–9.84 ng/μl, although purity values were inconsistent, with some negative A260/A280 readings.

Based on the quality of extracted DNA, the CTAB Wizard method was more effective for lipstick and serum matrices, while the PowerPrep™ kit showed superior DNA recovery from cream moisturiser despite purity challenges. These findings indicate that extraction efficiency is strongly matrix-dependent, and optimization is required for reliable detection of porcine DNA in complex cosmetic formulations.

3.2. Lard DNA Detection using qPCR

The detection of lard DNA in cosmetic matrices was assessed using different extraction methods and qPCR amplification. The outcomes for lipstick, serum, and cream moisturiser spiked with varying concentrations of lard DNA are summarized in Table 6.

Table 6. Lard DNA detection using qPCR

Lipstick		
Concentration of spiked lard DNA (%)	Extraction Method	
	CTAB Wizard	DNeasy® Mericon Food Kit
0	Not detected	Not detected
0.01	Not detected	Not detected
0.05	Not detected	Not detected
2	Not detected	Not detected
5	Not detected	Not detected
Serum		
	CTAB Wizard	Modified CTAB
0	Not detected	Not detected
0.1	Detected	Not detected
1	Detected	Detected
2	Detected	Not detected
5	Detected	Not detected
Cream Moisturiser		
	DNeasy® Mericon Food Kit	PowerPrep™ Gelatin DNA Extraction Kit
0	Not detected	Not detected
0.5	Detected	Detected
1	Detected	Detected
2	Detected	Detected
5	Detected	Detected

For lipstick, lard DNA was not detected at any of the tested spiking levels of 0.01%, 0.05%, 2% and 5% using either the CTAB Wizard or the DNeasy® Mericon Food Kit. The unspiked samples of 0% lard concentration and negative control also showed no amplification in the sample.

In serum, at spiking levels of 0.1%, 1%, 2%, and 5%, the CTAB Wizard method successfully detected lard DNA in the samples, whereas the modified CTAB method only yielded positive detection at 1% spiking. No amplification was observed in unspiked samples and negative control. These results indicate that the CTAB Wizard method was more reliable for DNA extraction from serum compared to the modified CTAB protocol.

For cream moisturiser, both the DNeasy® Mericon Food Kit and the PowerPrep™ Gelatin DNA Extraction Kit successfully detected lard DNA at all spiking levels from 0.5% to 5%. No amplification was observed in the unspiked samples and negative control.

4. Discussions

4.1. Quantification and Purity of DNA

Spectrophotometric analysis provided an initial indication of DNA yield and quality across the tested cosmetic matrices. Measurements at 260 nm reflect the concentration of total DNA present but are not specific to porcine DNA, meaning that elevated values cannot be directly interpreted as high quantity of target DNA. Similarly, the 260/280 nm absorbance ratio, typically expected between 1.8 and 2.0 for pure DNA, served as a guide for assessing contamination. Ratios below 1.8 suggest the co-extraction of proteins or polysaccharides, while ratios above 2.0 are indicative of RNA contamination.

The results demonstrated that acceptable purity values did not consistently correspond to successful downstream detection. For example, in lipstick samples, DNA concentration and purity fell within or close to the acceptable range, yet porcine DNA could not be amplified in qPCR. This highlights a key limitation of relying solely on spectrophotometric analysis as indicators to evaluate DNA quality, particularly in complex cosmetic products. DNA integrity and amplifiability are more critical for qPCR performance than absolute yield or purity values.

All extraction methods tested in this study were based on the cetyltrimethylammonium bromide (CTAB) principle with specific modifications. Methods incorporating purification steps and column-based recovery, such as the CTAB Wizard and the DNeasy® Mericon Food Kit, generally produced DNA within the acceptable purity range, especially for lipstick and

serum samples. However, this advantage was not observed consistently in cream moisturiser samples, where impurities appeared to persist despite the purification steps. This finding supports previous studies reporting that matrix-specific properties, such as fat and emulsifier content, may interfere with DNA isolation and compromise downstream amplification (Sofiyani *et al.*, 2022).

Taken together, these observations indicate that while spectrophotometric analysis provides useful preliminary insights into DNA extraction performance, it is not sufficient to predict the success of qPCR detection. Instead, DNA integrity and compatibility of the extraction method with the cosmetic matrix play a more critical role.

4.2. Lard DNA Detection using qPCR

The ability of extracted DNA to serve as a suitable template during qPCR amplification is the crucial measure of extraction method performance. In this study, neither the CTAB Wizard nor the Dneasy® Mericon Food Kit were able to detect lard DNA in lipstick samples, despite yielding measurable concentrations and acceptable purity ratios. This outcome underscores that high DNA yield and acceptable purity do not necessarily guarantee amplifiability in qPCR.

Lipstick represents a particularly challenging cosmetic matrix due to its chemical complexity. Ingredients such as waxes, colourants, metal ions, and polysaccharides are known to act as PCR inhibitors, directly interfering with polymerase activity and amplification efficiency (Abboosh *et al.*, 2023; Kim *et al.*, 2018). In addition, cosmetics such as lipstick undergo harsh processing steps that can degrade DNA into fragments smaller than the qPCR amplicon size (S Abd-Gani *et al.*, 2019). Degraded DNA may still contribute to spectrophotometric concentration and purity values but is unsuitable for successful amplification. This explains the inconsistent inconsistency during detection of porcine DNA in lipstick samples, even when spiked at levels as high as 5%.

In contrast, serum samples highlighted the benefit of purification steps and column-based recovery. The CTAB Wizard successfully detected porcine DNA, whereas the modified CTAB method only yielded detection in one low-spike sample (1%). This suggests that purification steps are critical for removing inhibitors and producing DNA fragments compatible with amplification.

Interestingly, cream moisturiser samples demonstrated successful amplification across all spiking levels when extracted with the Dneasy® Mericon Food Kit and the PowerPrep™ Gelatin DNA Extraction Kit, even though DNA concentration and purity values were

relatively low. This suggests that these methods prioritise the recovery of extracted DNA rather than maximising the DNA yield. Previous studies have reported similar findings: Sofiyani *et al.*, (2022) demonstrated that double spin-column approaches, such as the PowerPrep™ Gelatin DNA Extraction Kit, consistently recover DNA fragments suitable for amplification, while Kim *et al.*, (2018) identified the PowerPrep™ kit as particularly well-suited for cosmetic products.

Collectively, these findings highlight that the success of qPCR detection is influenced more by the elimination of inhibitors and the preservation of amplifiable DNA fragments than by achieving high DNA concentration or purity values. The consistent detection in cream moisturiser, compared to the failure in lipstick, illustrates the strong matrix-dependent challenges in DNA-based *halal* authentication of cosmetics.

Taken together, the results from DNA quantification and qPCR detection highlight a critical distinction between DNA yield and amplifiability. While spectrophotometric measurements indicated that acceptable concentrations and purity ratios could be obtained, particularly in lipstick and serum samples, these values did not always translate into successful detection. Instead, the effectiveness of porcine DNA detection depended largely on the extent to which extraction methods removed inhibitors and preserved intact DNA fragments compatible with amplification. These observations emphasise the need to evaluate DNA extraction methods not only by concentration and purity metrics but also by their downstream performance in qPCR, especially when applied to complex and highly processed cosmetic matrices.

5. Conclusions

This study aimed to assess the efficiency of various DNA extraction methods for the detection of porcine DNA in cosmetic products with complex matrices. Four extraction methods were evaluated: the Dneasy® Mericon Food Kit, PowerPrep™ Gelatin DNA Extraction Kit, CTAB Wizard, and a modified CTAB protocol.

The findings demonstrated that all four methods are capable of extracting and detecting porcine DNA; however, their performance varied depending on the cosmetic matrix. Methods incorporating purification steps and the use of purification columns, particularly the CTAB Wizard and PowerPrep™ Gelatin DNA Extraction Kit, yielded higher-quality DNA that was more suitable for qPCR amplification. By contrast, matrices rich in waxes, pigments, or other inhibitory compounds posed significant challenges, highlighting the impact of product formulation on DNA recovery and detection.

Overall, this research emphasizes that there is no universal extraction method applicable to all cosmetic products. Instead, method selection should be optimised to the matrix composition of the product being analysed. These insights contribute to the advancement of reliable DNA-based detection in the *halal* cosmetic industry, supporting efforts to strengthen product verification and consumer confidence.

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