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# Food Safety Monitoring: The Use of Specific Swine Detection Methods to Ensure Halal Authenticity

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## ABSTRACT

In the past few decades, Halal meat has had growing sales with Muslim communities totalling nearly 25% of the world population. The qualification of Halal, permitted as per Islamic Shari'ah, addresses attributes that refer to the method of production and establishes that products must be free of any prohibited ingredients, such as pork, animals slaughtered improperly and other intoxicants. Despite preventive measures, food industries might fail to produce food which is not correctly described and may be contaminated with pork derivatives. Analytical tests in meat have increased in recent years due to the discovery of species adulteration in processed products. To ensure Halal authenticity, food safety enforcement authorities perform controls at each stage of the agri-food chain, and Halal entities are responsible of certifying goods apt for consumption by Muslims through coherent measures and adequate analytical monitoring. Our laboratory analysed a total of 898 samples supposed to be Halal using a highly sensitive analytical method (sensitivity > 0.0005%) to discover that a significant proportion of the samples analysed presented traces of pork DNA. Such small amounts of pork DNA might end up adulterating the final products due to accidental contamination during processing, thus rendering it Haram, or non-permitted.

The presented study highlights the importance of implementing specific and sensitive analytical surveillance methods to ensure the authenticity of Halal products.

## INTRODUCTION

In the past few decades, Halal meat has had growing sales with Muslim communities totaling nearly 25% of the world population. The qualification of Halal, permitted as per Islamic Shari'ah, addresses attributes that refer to the method of production and establishes that products must be free of any prohibited ingredients, such as pork, animals slaughtered improperly and other intoxicants.

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## MATERIALS AND METHODS

A total of 898 products submitted between January 2018 and June 2019 by multiple meat manufacturer were included in the study.

For genetic analysis, DNA was extracted from each meat sample using a commercial **GMO Extraction kit** (Thermo Fisher Scientific). To ensure optimal representativeness of the sample, 200 g of raw meat were homogenized from which 10 g were incubated in lysis buffer, proteinase K and RNase reagent according to the manufacturer's instructions.

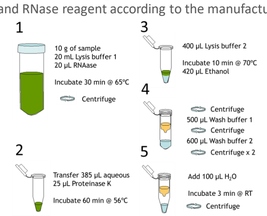


**Fig 1. DNA extraction protocol to ensure a high quality and purity DNA sample.**

The quality and quantity of the purified DNA sample was studied by spectrophotometry. The analytical monitoring was performed by Real-Time PCR using the Applied Biosystems™ 7500 FAST Real-Time PCR System Thermo Scientific™ RapidFinder™ Pork ID kit (Thermo Fisher Scientific) and RapidFinder™ Halal ID kit (Thermo Fisher Scientific). The swine amplification systems target highly specific mitochondrial DNA regions, which confer great specificity and sensitivity (limit of detection established at 0.01% and 0.0005%, equivalent to 5 ppm), respectively.

For this, a total amount of 50 ng of total DNA were used to set up the PCR reaction. All samples were extracted and analyzed in duplicate.

## RESULTS

Our results indicated that all DNA samples were optimal for the genetic analysis. The **GMO Extraction kit** (Thermo Fisher Scientific) is optimal for low-target detection in assays that require High sensitivity as it processes over 20-times more sample than most commercial kits. In addition, it yields high DNA concentration (>100 ng/µL) and purity suitable for any PCR-based downstream assays.

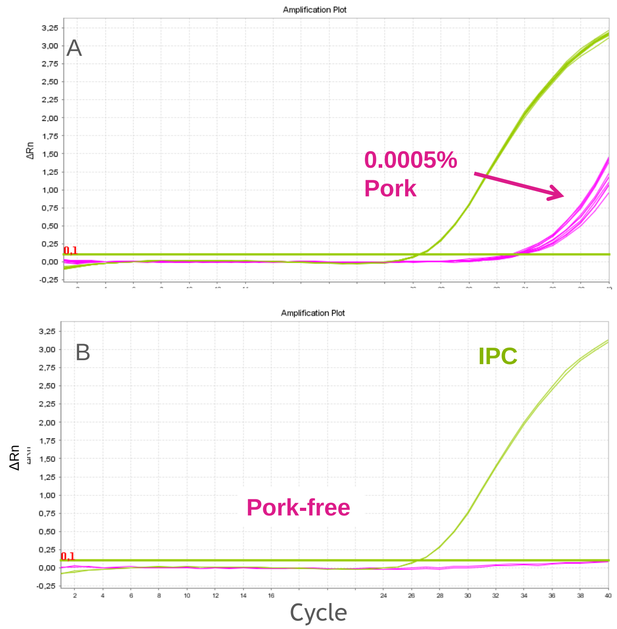


**Fig 2. Real-Time PCR amplification plot. A) Detection results obtained from a DNA sample containing 0.0005% (5 ppm) swine DNA. Swine DNA is labelled with FAM, and the internal positive control (IPC) with VIC. The IPC consists of a synthetic DNA region directed to function as a PCR control to confirm the correct set up and functioning of the PCR; B) Swine-free sample suitable for Halal labelling.**

The **RapidFinder Halal ID kit** is a highly sensitive system capable of detecting 5 ppm of pork DNA. This sensitivity allows meat manufacturer to reliably label and trace the authenticity of the sample. Diagnostic detection of pork DNA using Real-Time PCR is interpreted based on a cycle threshold ($C_t$) value[1]. The cut-off value is stablished on 0.0005% above which a $C_t$ value is deemed false.

Overall, 13.5% of the samples contained pork DNA. Among them 35 were only detected by the Halal-specific assay with a sensitivity of 0.0005% (5 ppm).

Analytical surveillance confirmed that 87% of the Halal products were free of pork. Overall, 120 samples contained traces of pork, all efficiently detected by the Pork ID Kit with a cut-off at 0.01%.

Follow-up monitoring informs the Halal product manufacturers of a breakage in the cleaning and decontamination process in the production chain enabling them to seek solution to mitigate cross-contamination with pork-containing products.

The CODEX ALIMENTARIUS COMMISSION provides General guidelines for the use of the term "Halal", and legislation is now available in various territories. For instance, the EU Commission released a recommendation indicating follow-up controls shall be performed [2,3] and the removal of debris and product changes must be ensured to comply the acceptability limit of <1% accidental contamination, guidelines. However, Halal requirements vary between country authorities.

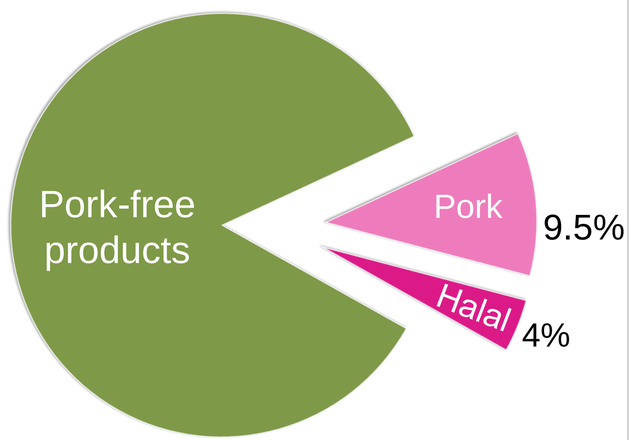


**Fig 3. Graphic representations of the 520 Halal products analysed. Light pink, samples detected with the RapidFinder Pork ID kit; Dark pink, samples detected with highly sensitive (5 ppm) the RapidFinder Halal ID kit.**

## CONCLUSIONS

The presented study indicates that a highly sensitive analytical tool is capable of detecting traces of swine DNA to ensure the authenticity of Halal products. Both genetic assays successfully detected swine DNA at concentrations below the EU Commission recommendation to prevent food fraud.

In conclusion, the present study highlights the effectiveness of implementing analytical surveillance to ensure the authenticity of food products by minimizing accidental contamination.

## REFERENCES

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