

APPLICATION OF MULTIPLEX PCR COUPLED WITH DNA STRIP FOR DETECTING FIVE NON-HALAL ANIMALS IN FOOD PRODUCTS

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Lab Screening of Forbidden Substances

147

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Research Paper

Postmarket Laboratory Surveillance for Forbidden Substances in Halal-Certified Foods in Thailand

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ABSTRACT

Limited information exists regarding adulteration of Halal-certified food by substances forbidden under Islamic law (Haram substances). This study was conducted using forensic laboratory testing to investigate the prevalence of this type of adulteration. In this large-scale survey of Halal-certified food products randomly collected from markets in Thailand, 4,829 food samples from 10 food groups were tested in the laboratory for four potentially Haram substances: porcine DNA, porcine fatty acids, ethanol, and hydroxyproline (gelatin). No samples were adulterated with porcine DNA or fatty acids. However, 62 samples (approximately 1.3%) were positive for ethanol (>0.5% for non-naturally fermented products and >1% for naturally fermented products). The hydroxyproline concentration in the samples was compared with that of a negative control. Gelatin, as indicated by the presence of hydroxyproline, was the major suspicious substance found in these products. Further investigations are required to determine whether the gelatin is of Halal origin. These results from this first large-scale postmarket surveillance of Halal-certified food products for forbidden substances reveals the important role of forensic laboratory testing for supporting Halal supervision and certification. These findings provide useful information for government agencies seeking to encourage Halal compliance by food enterprises and for Muslim consumers and Halal food importers and exporters.

HIGHLIGHTS

- A total of 4,829 Halal food samples were tested for four substances forbidden by Islamic law.
- Of the total analyzed samples, 1.3% were questionable and needed further investigation.
- Gelatin was the major suspicious substance with concerns for its Halal origin.
- Laboratory testing may play an important role in preserving the Halal integrity of foods.

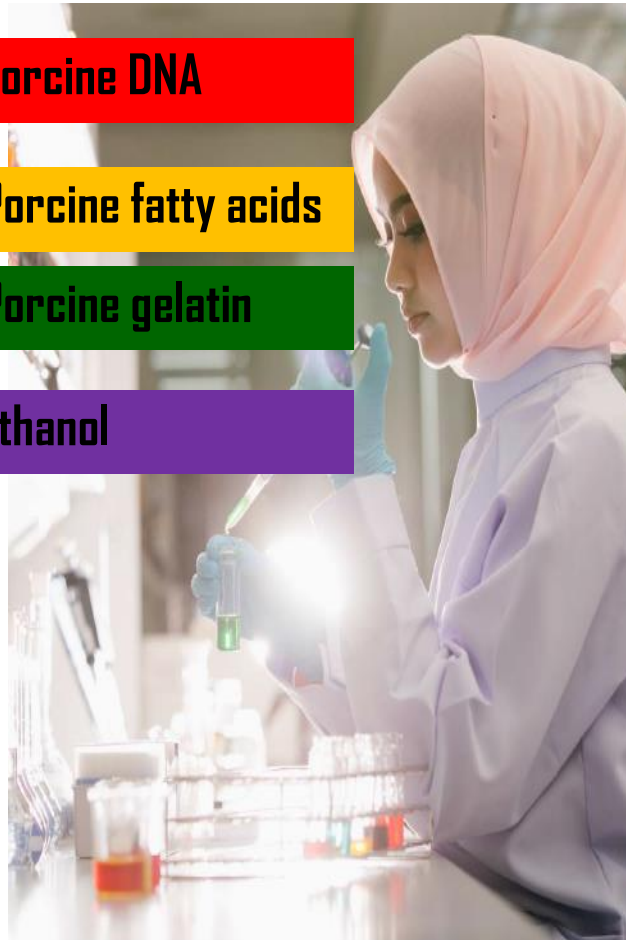
Key words: Adulteration; Food protection; Food safety; Halal food product; Haram

Porcine DNA

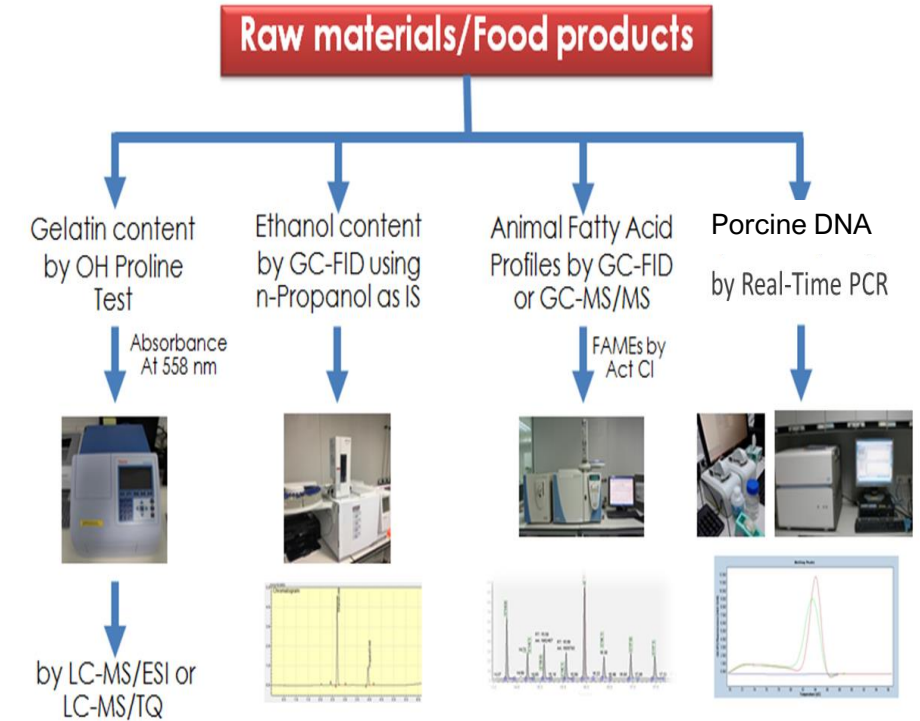
Porcine fatty acids

Porcine gelatin

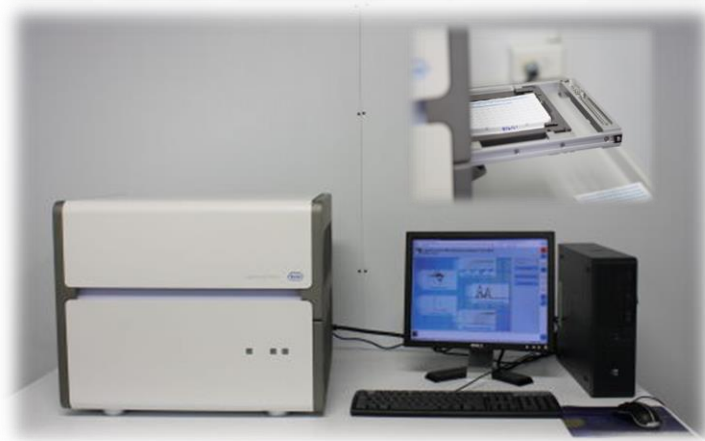
Ethanol



Routine Lab Screening of Haram Adulteration



Detection of porcine DNA by Real - Time PCR



Meat adulteration

Cat meat being sold as mutton in roadside eateries

Manish Raj@timesgroup.com
Chennai: Meat lovers beware! The succulent lamb chops or spicy roasted mutton pieces at a roadside eatery might be prepared from cat meat.
 "In the past few months, there has been a spurt in demand for cat meat," says G Arun Prasanna of People for Cattle in India (PCCI). Volunteers have rescued around 40 stolen cats — meant to be slaughtered for meat — from Arani, Agumaram, and Red Hills in the last six months.

Red Hills, followed by Pallavaram (14). Recently, a large number of carcasses and cat skins were recovered at Kotturpuram gypsy colony. "A total of seven police complaints have been registered so far," said a police officer.
 A large polythene bag of cat meat weighing between 2kg and 3 kg costs around ₹100. It is supplied to small vendors and biryani stalls across the city. They are then sold to unsuspecting customers as mutton. (Name changed) from Shankar, a former teenage help at Pallavaram.
 Explaining the modus operandi, S Shanmugham, another volunteer said, the gypsies target cats near MRTS stations and bus depots. These cats are caught with nets, drugged and stuffed in gunny bags. There is another facet which fuels the demand for cat meat. Many people believe that cat meat is an aphrodisiac and has several medicinal properties. Quacks prescribe cat meat for impotence, asthma, and other ailments. Street food activists, including PCCI, have been active in sensitising the public. Attempts to contact heads of gypsy community proved futile. Volunteers conducted a sensitisation camp at Poomalloor gypsy colony in January. "We plan to conduct more camps as according to Food Safety and Standards Authority (FSSAI) cat meat is unfit for human consumption," said



WATCH WHAT YOU EAT



Rat meat sold as lamb in latest China food scandal

Rodent-peddling Jiangsu gang broken up in government crackdown on adulterated products



Police seize more than 100 monkeys allegedly trafficked in illegal meat trade

Published on Friday, May 28, 2021 16:10
 By Tanutarn Thawan



<https://timesofindia.indiatimes.com/city/chennai/Cat-meat-being-sold-as-mutton-in-roadside-eateries/articleshow/46336721.cms>

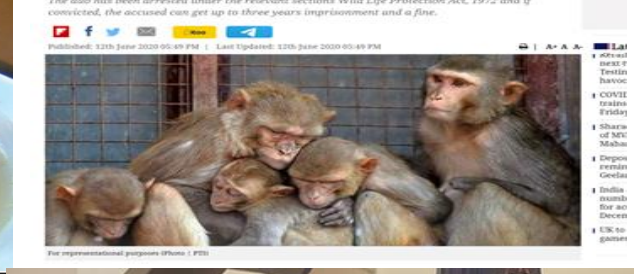
Dog meat: First Cambodian province bans sale and consumption



Mystery Meats



Two held for hunting monkey, eating its meat in Maharashtra's Pune district



ระวัง เนื้อวัวปลอมระบาดหนัก



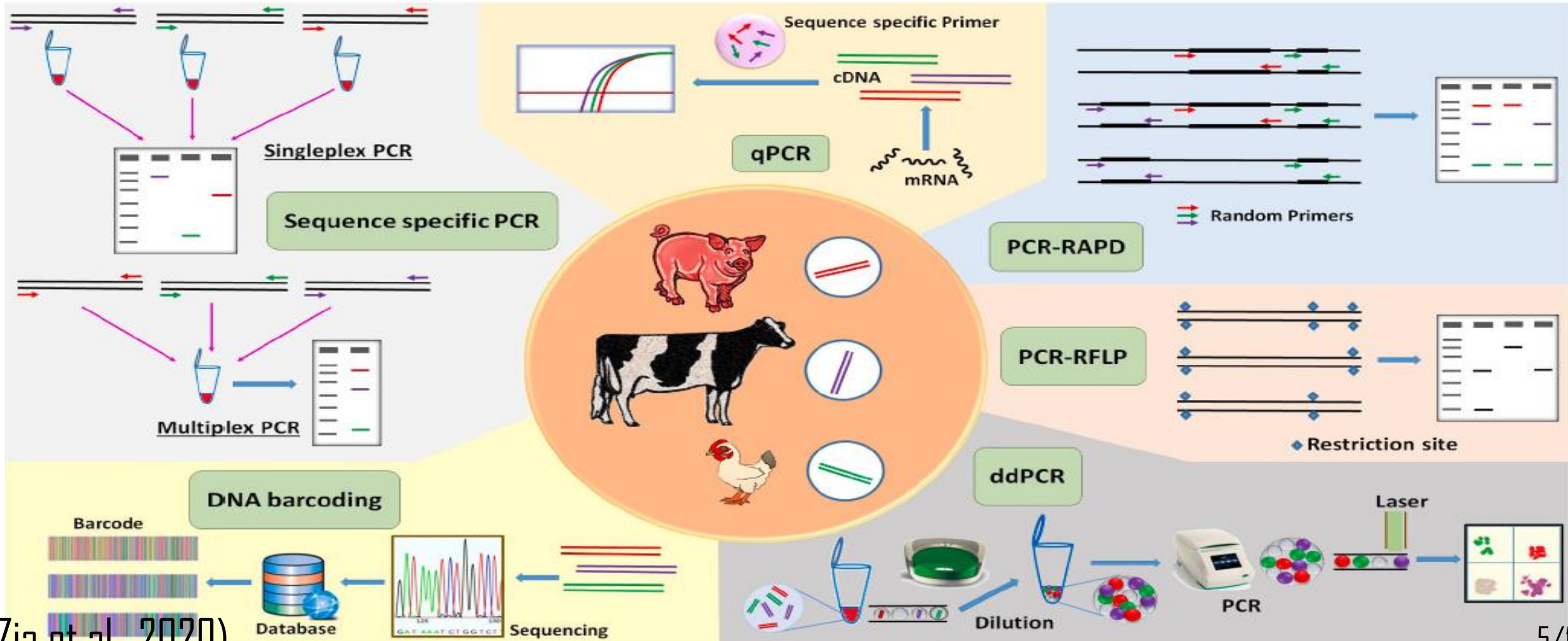
Meat matters: Feasting on dogs and donkeys



(Ali et al., 2015; Ulca et al., 2013).



DNA based methods



The powerful DNA techniques

To become a major Halal food exporter, building up confidence for Thailand in the world stage by having a tool to inspect halal food quality is thus crucial.



มาตรฐานสินค้าเกษตรและอาหารแห่งชาติ

มกษ. 8400-2550

THAI AGRICULTURAL COMMODITY AND FOOD STANDARD

TACFS 8400-2007

อาหารฮาลาล

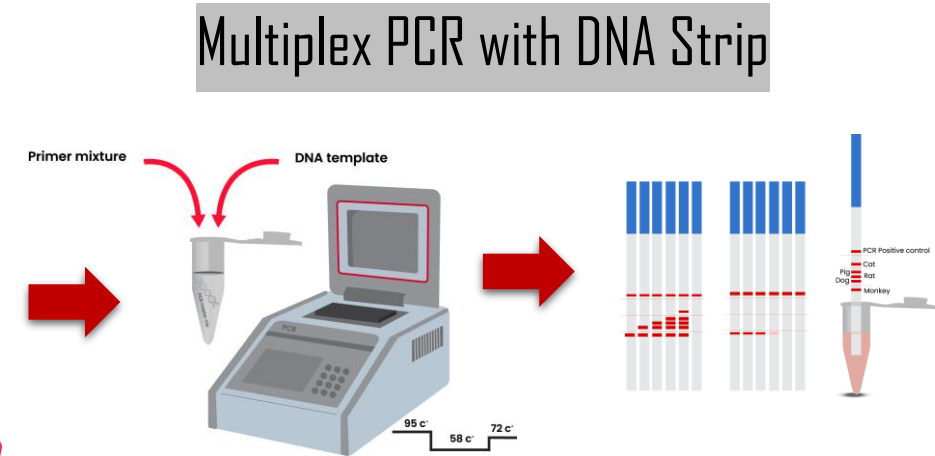
HALAL FOOD

สำนักงานมาตรฐานสินค้าเกษตรและอาหารแห่งชาติ

กระทรวงเกษตรและสหกรณ์

ICS 67.040

ISBN _____



- Simple
- Fast
- Low-cost

Multiplex PCR with DNA strip

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Modern on-site tool for monitoring contamination of halal meat with products from five non-halal animals using multiplex polymerase chain reaction coupled with DNA strip

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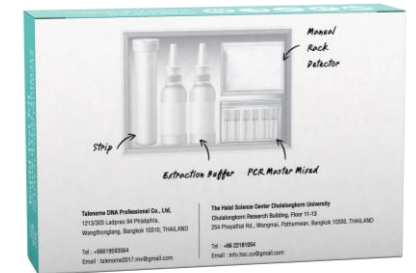
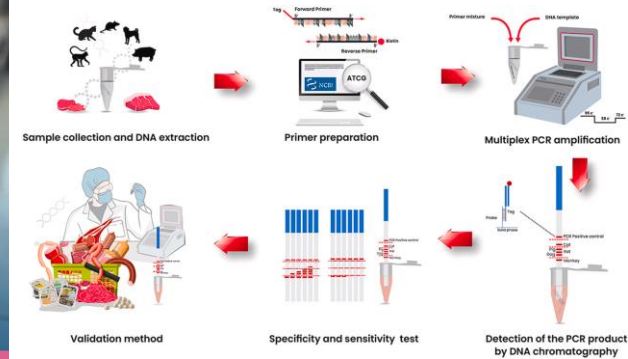
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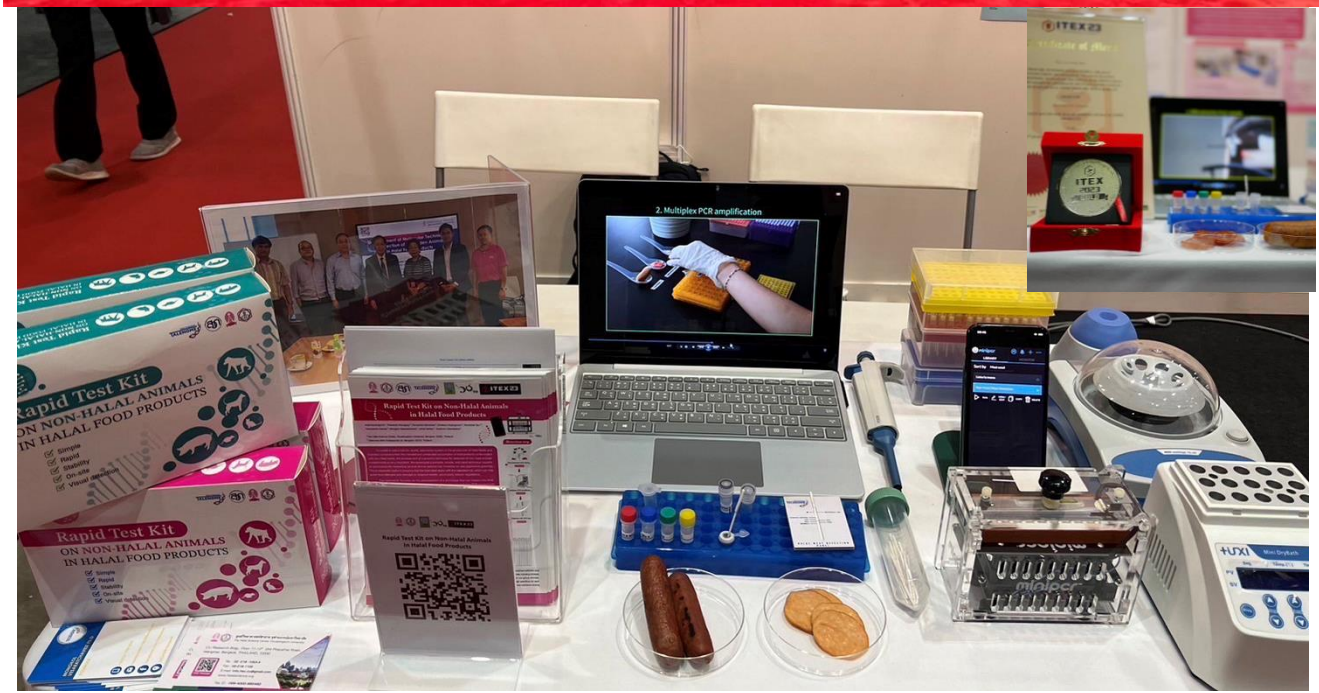
Meat adulteration
Meat authentication
Multiplex PCR
DNA strip
Halal-certification
Halal standard

ABSTRACT

Meat adulteration is a persistent problem in the food industry in many countries. This unfair practice can potentially not only affect the economy of a country but also the confidence and health of its citizens. Additionally, it can aggravate allergies or damage religious beliefs (e.g., Muslims). The aim of this study was to develop a simple and rapid technique for the on-site inspection of several targets using multiplex polymerase chain reaction (PCR) coupled with DNA strip for detecting contamination with products from five non-halal animals (monkeys, dogs, rats, pigs, and cats) in foods certified to be halal. Species-specific primers were retrieved from the mitochondrial genes of each non-halal animal and modified with a tag sequence and biotin. The selected primers showed high specificity for each non-halal animal. The PCR products obtained after amplification were detected using DNA strip. The limit of detection ranged from 0.01 ppm to 1 ppm (dogs, 1 ppm; cats, 0.1 ppm; pigs, 0.01 ppm; monkeys, 0.01 ppm; and rats, 0.01 ppm). Beef meatballs spiked with non-halal meat in different ratios were prepared to validate the developed technique, which presented 100% accuracy in detection. In addition, the developed technique was applied to verify 115 samples of meat products commercially available in Thailand. Sixteen samples were contaminated with pig DNA, but contamination with the DNA of other non-halal animals was not found. Therefore, this technique has high potential for on-site monitoring of non-halal animal contaminants in halal products in Thailand.

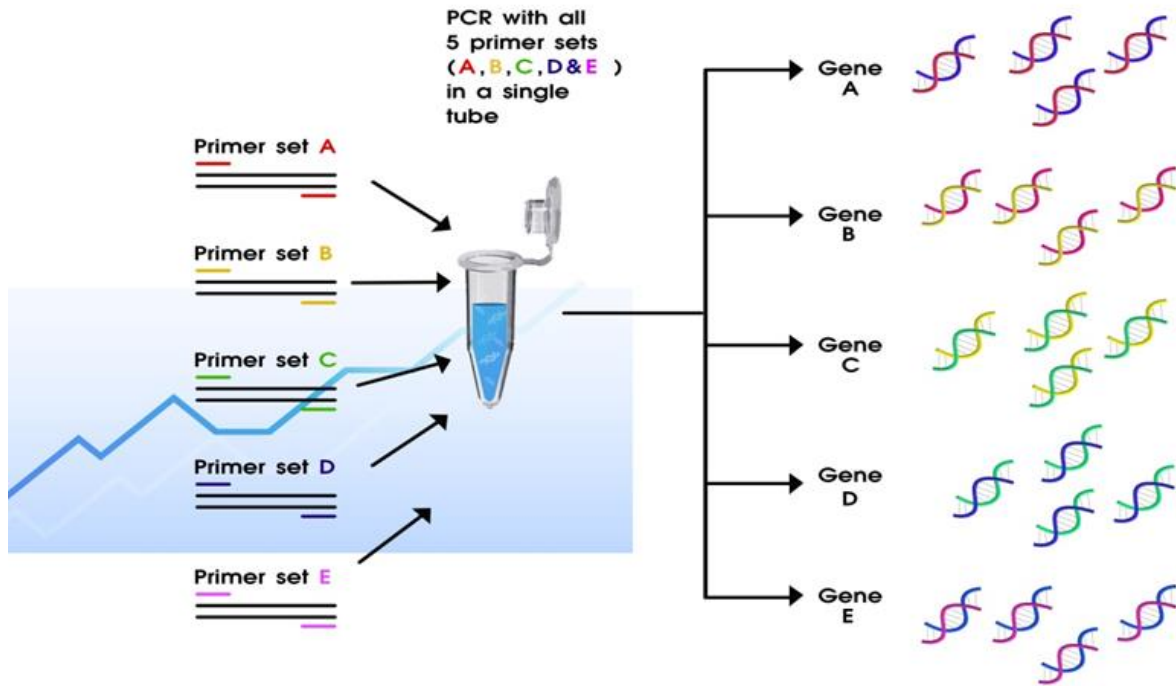
(Denyinghot et al., 2022)





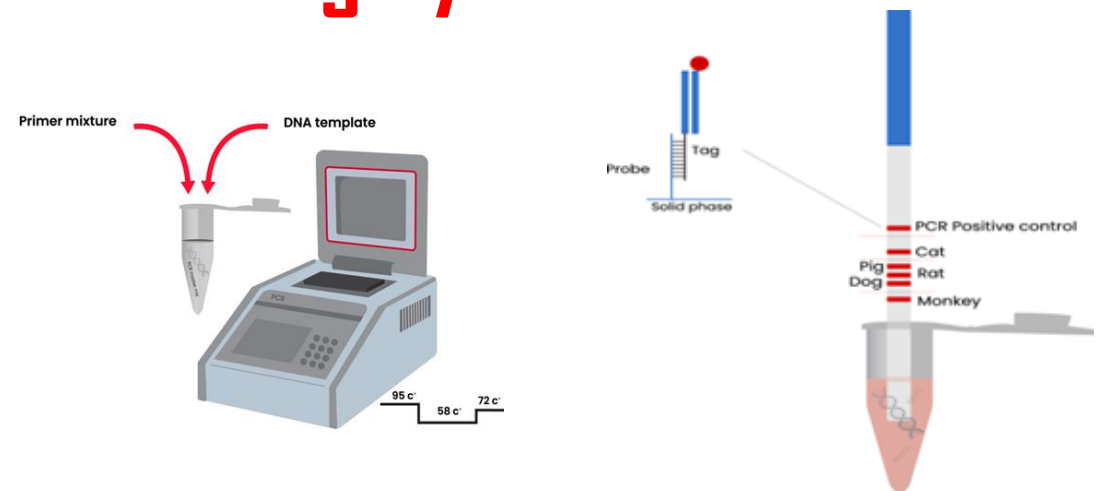
**Gold medal award in ITEX 2023 ,
KL , Malaysia on May 12 , 2023**

Multiplex PCR with DNA strip



Multiplex PCR

- ✓ cost effective
- ✓ saves times
- ✓ highly accurate



5 non-halal animals detection kit



**Rapid Test Kit
ON NON-HALAL ANIMALS
IN HALAL FOOD PRODUCTS**

- ✓ Simple
- ✓ Rapid
- ✓ Stability
- ✓ On-site
- ✓ Visual detection

Manual Rack Detector

Strip *Extraction Buffer* *PCR Master Mixed*

HALAL MEAT DETECTION PANEL

POSITIVE CONTROL
CAT
BAY
MONKEY
PIG
DOG

----- REFERENCE LINE

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A simple and rapid technique for Detecting contamination from 5 non-halal animals in food 10/17

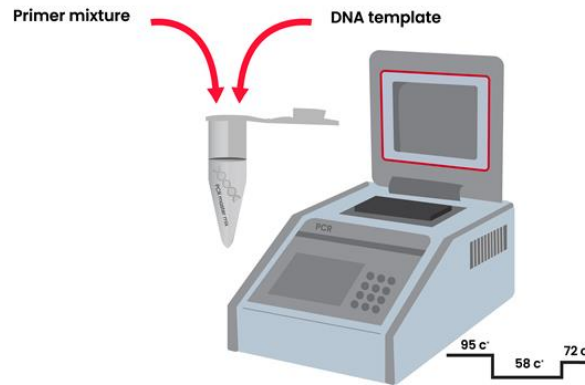
5 non-halal animals detection kit

3 Steps for user



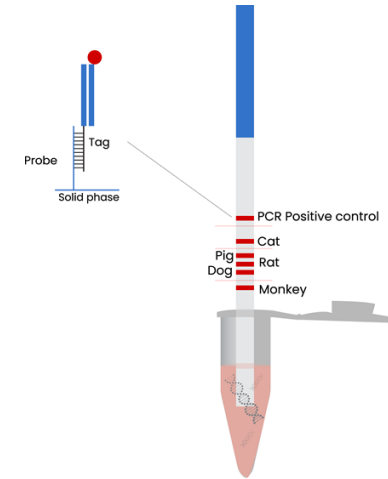
1

DNA extraction
20 Mins



2

Multiplex PCR amplification
60 Mins

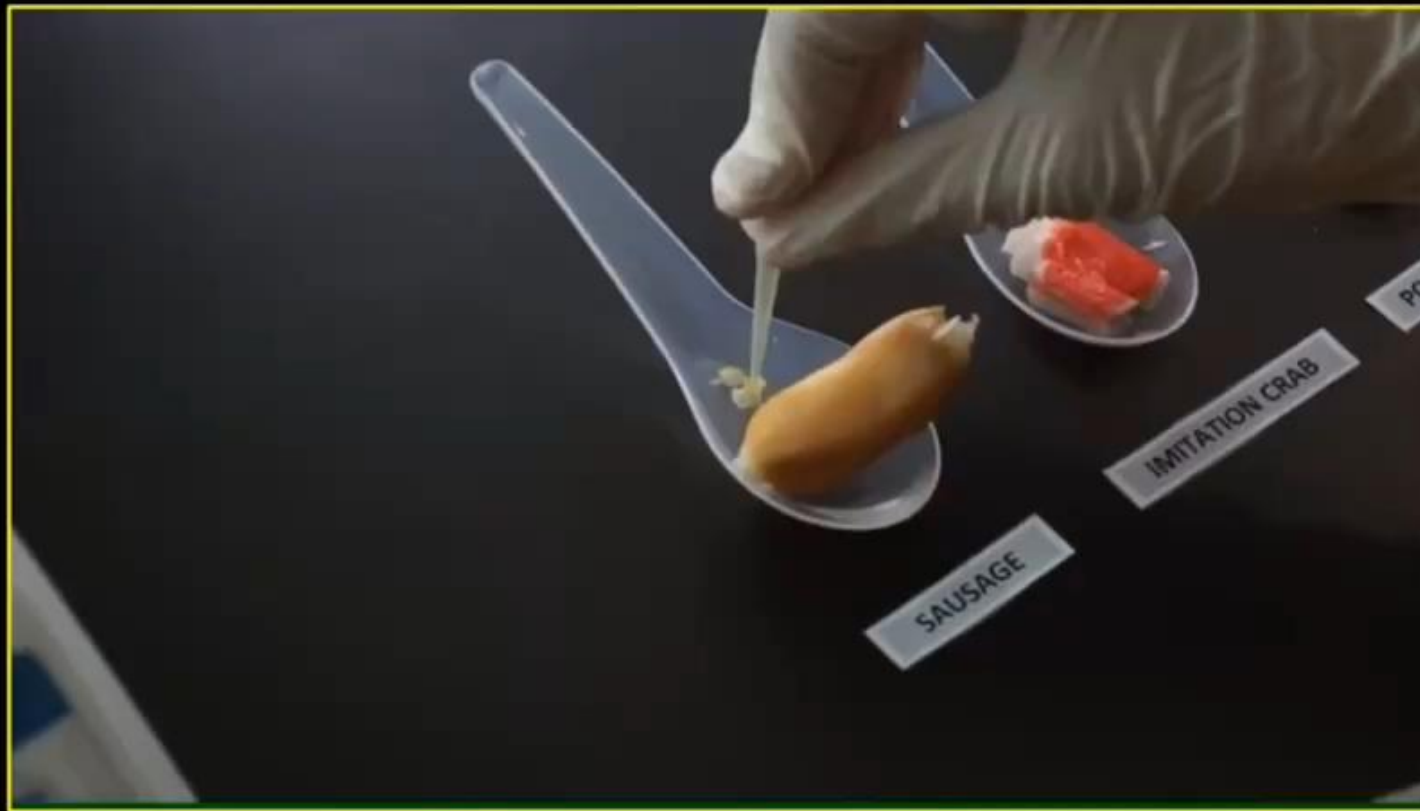


3

Detection with DNA strip
15 Mins

5 non-halal animals detection kit

1. DNA extraction from sample

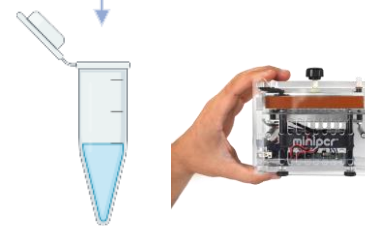
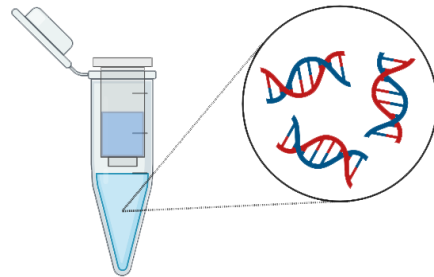
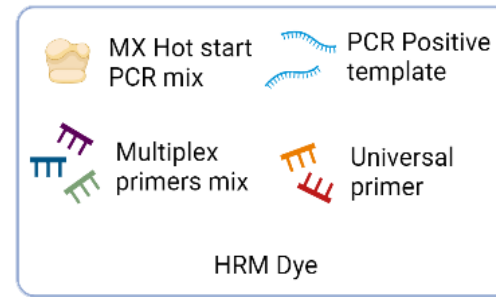
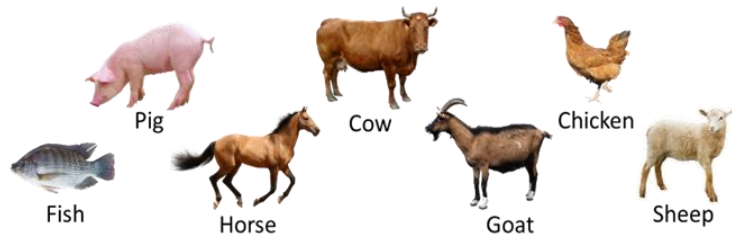


5 non-halal animals detection kit

- 1 Equipment **2,000 USD**
- 2 Price per sample **15 USD**
- 3 Analysis time **90 Mins**
- 4 Sample volume **10 milligram**
- 5 Portability **Yes**
- 6 User expertise **Basic (scientific based)**
- 7 Shelf life **2 years at chill temperature**



Meat authentication platform



Food products

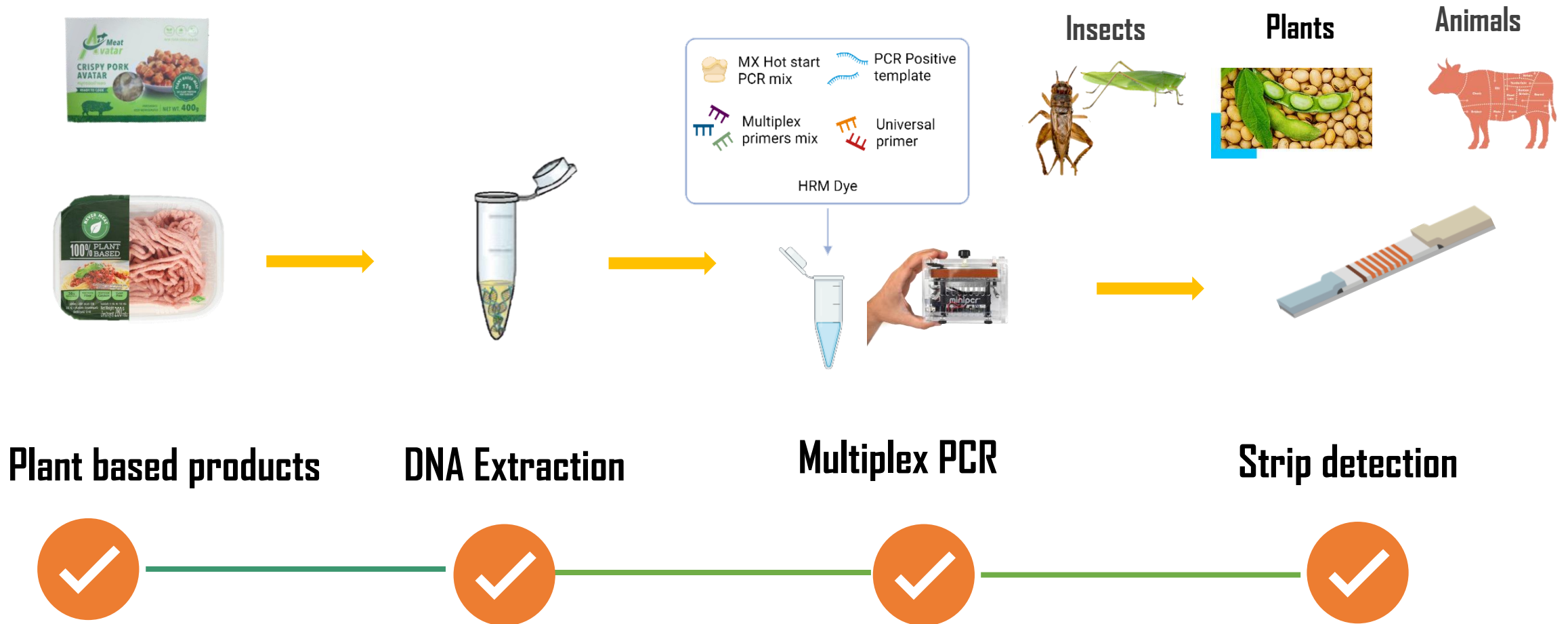
DNA Extraction

Multiplex PCR

Strip detection



Plant based detection platform



Summary



- This is innovative tool that can ensure halal food producers and consumers that there was no non-halal animal contamination in raw materials and food products.
- This tool can be employed as a crucial step to support halal certification and could apply for onsite detection.
- To the best of our knowledge, this is the first study of a successful development of techniques on simultaneous detection five non-halal animal contaminations in halal food products in THAILAND .

Question & Answer



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