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# A New Technique to Detect Pig Hair by Immunochromatographic Rapid Test

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**ABSTRACT**: Pig hair can be used as brush to smear bread/cookies/other food or to clean devices for example a baking pan, so the food can be contaminated by haram stuff. As a brush, pig hair has no root of hair. If root of hair with some blood and skin are examined, DNA typing usually can be performed by moleculer biological technique. But, hair ends of the brush contain a lot of protein. Thus, the objective of the research is to develop a new technique to detect pig hair. The samples were pig hair ends without their root. The protein of the hair was biochemically extracted, and then protein was detected by immunochromatographic rapid test kit. Hair was rinsed by SDS 2% and PBS, and digested by H2SO4 10%. The protein content was estimated by Nynhydrin 5%, and was tested for pig species by Xema pork rapid test. The result showed that the protein could be collected, and the sample was detected positively as pig species.

**Keywords**: pig, hair, protein, extraction, Xema rapid test

## INTRODUCTION

Paint brush can be made of pig hair and used to clean devices for example a cake baking pan or to smear bread/cookies/other food. The food will be contaminated by pig hair and then declared to be haram for moeslems. Most Indonesian are moeslems, so a method for detection of species hair of brush should be created. The study was an initial research by detection of origin pig hair.

Hair consists of three concentric layers. The innermost layer is known as the medulla; the middle part of the hair, known as cortex;and the outer is known as the hair cuticle (Hughes, 2013). The most content of cortex is keratin and keratin-associated protein (KAPs). The KAP proteins interacted with each other and preferentially bound to hair keratins, but not to epithelial keratins (Fujikawa *et al*., 2012). Hair is a part of the skin that grows out of a structure known as the hair follicle. The length of a hair extends from the root, continues into a shaft, and terminates at the tip. The hair shaft is the part of the hair that protrudes out of the scalp. The hair shaft is part of the hair that is usually used as materials for brushes. The shaft does not consist of any nuclear DNA for a DNA test (Fujikawa *et al*., 2012). Some new techniques which allow DNA typing to be performed without a root need a lot of hairs.

In hair being absence of a root with skin or blood, color and structure (morphology) of hair is used to diagnose in the forensic feature. But, hair products, such as paint brush might be changed in color and structure. Another analysis, proteomic analysis based on protein is an alternative way to study on characterization of hair (Rouse and .Van Dyke, 2010). Some protein extraction methods have been performed with solution of Tris-HCl, thiourea, urea,and mercaptoethanol (Fujii and Li, 2008; Han *et al*., 2007; Nakamura *et al*., 2002). The objective of the research is to create a new technique to detect pig hair by extraction of protein by SDS and tested for pig species by Xema pork rapid test.

## MATERIALS AND METHODS

Pig hair ends (hair shaft) were examined. The methods of the research used biochemically protein extraction, and the detection of pork species was carried out by immunochromatographic rapid test kit. The protein extraction methods were modified from an experimental procedure of Lee *et al*. (2006). The hair cut of 1 g in weight were rinsed in 10 ml solution of 2% SDS for ten minutes and drained by filtered paper. The hair was then immersed in 10 ml solution of 2% SDS, and the supernatant was discarded. The hair in 10 ml of phosphate buffer saline (PBS) and 10 ml solution of 2% SDS was incubated at 65 °C for overnight. On the next day, the hair was homogenized by magnetic stirer for an hour at room temperature. Soluble and insoluble materials were separated by centrifugation at 8000 rpm for 5 minutes. Supernatant was collected into a tube. Protein was then digested with 5 ml solution of 10% sulfuric acid and put into waterbath at 40 °C for an hour. The bubble was neutralized by dropping a solution of 50% ammonium bicarbonate (NH4 HCO3). The tube was kept into freezer. The solution in the bottom was analyzed for pork species by pork rapid test (Xema, Malaysia). The protein content could be estimated by a reaction with 5% ninhydrin after dropping ammonium bicarbonate.

## RESULTS AND DISCUSSION

The extraction produced two layer of solution in which protein was shown to be in the bottom (Figure 1) and the pork rapid test resulted in 2colour line considering positive (Figure 2). The extraction was modified from the method used to analyze human hair shaft by Lee *et al*. (2006) to be a simple extraction method. The modified methods just need SDS without DTE and take 3 days to do extraction process, while Lee's method need at least 10 days (Table 1). According to Lee *et al*. (2006), protein identified from soluble fraction consists of mostly disulfide-cross-linked keratins and KAPs. Keratin associated proteins of human hair shaft served to distinguish individual profiles of Caucasian, African-American, Korean, and Kenyan (Laatsch *et al*., 2014). That of pig hair may also serve to distinguish hair species by immunoassay,such as a Xema pork rapid test.

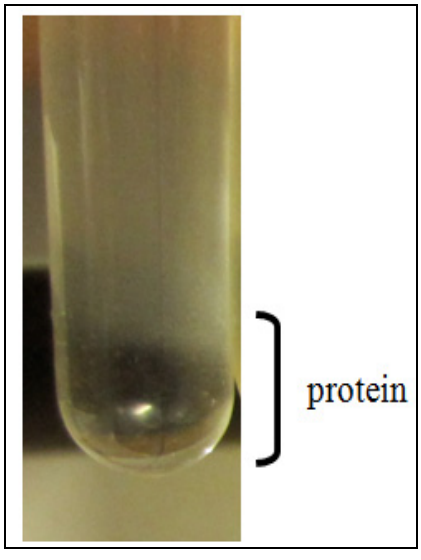


Figure 1. Extraction of protein

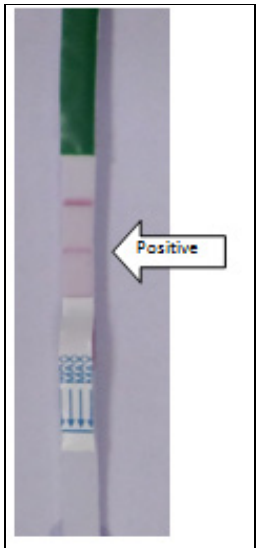


Figure 2. Xema pork rapid test

**Table 1**. The differences between Lee's and the modified extraction methods.

| The method according to Lee *et al*. (2006) | The modified method |
|---|---|
| Extraction:<br>- 40 mg of human hairs were rinsed in 5 ml solution of 2% SDS, 50 ml solution of sodium phosphate (pH 7.8).<br>- It was drained, then immersed in 5 ml solution of 2% SDS, 50 ml solution of sodium phosphate (pH 7.8), and 20 ml solution of DTE.<br>- The mixture was incubated overnight at 65 °C, then pulverized by magnetic stirrer for an hour at room temperature<br>- The soluble and insoluble materials were separated by centrifugation.<br>- The insoluble material was resuspended in a solution of 2% SDS, 50 ml solution of sodium phosphate (pH 7.8), 20 ml solution of DTE.<br>- The insoluble material was then incubated overnight at 65 °C; and then extracted as the procedure before ( five extractions). | Extraction:<br>- 1 g of pig hairs were rinsed in 10 ml solution of 2% SDS for 10 min.<br>- It was drained by filter paper, then immersed in 10 ml solution of 2% SDS.<br>- The hairs in PBS and 10 ml of 2% SDS were incubated at 65 °C for overnight, then pulverized by magnetic stirrer for an hour at room temperature.<br>- The soluble and insoluble materials were separated by centrifugation at 8000 rpm for 5 minutes.<br>- The supernatant was then collected into a tube. |

Xema pork rapid test is immunoassay technique that can be used in the field of food technology. The principle of this technique is soluble antigen (usually a protein) diffused to the antibody. If the antibodies is in accordance with antigens, then the reaction is indicated by a line of sediment (Ahmad, 2005). In this research showed that protein of pig hair shaft and antibodydiffuse into each other forming a line.

## CONCLUSIONS

The conclusion showed that protein could be collected by a modified extraction method, and the pig hair shaft was detected positively.

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