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Technical note

The use of immunochromatographic rapid test for soft tissue remains identification in order to distinguish between human and non-human origin

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ABSTRACT

Clear identification of soft tissue remains as being of non-human origin may be visually difficult in some cases e.g. due to decomposition. Thus, an additional examination is required. The use of an immunochromatographic rapid tests (IRT) device can be an easy solution with the additional advantage to be used directly at the site of discovery. The use of these test devices for detecting human blood at crime scenes is a common method. However, the IRT is specific not only for blood but also for differentiation between human and non-human soft tissue remains. In the following this method is discussed and validated by means of two forensic cases and several samples of various animals.

1. Introduction

Occasionally, differentiating between human and non-human remains may be part of a forensic investigation. The excavation of bones at construction sites is not uncommon. For example, during construction at the Potsdamer Platz in Berlin, which was a major target area during World War II, a large number of human bone fragments was discovered. Among the human remains, several animal bones were found and sorted [1]. Animals can also be involved in mass disasters, and the disaster victim identification (DVI) process requires discriminating between human and non-human remains [2,3]. Additionally, hunters or hikers often encounter animal carcasses and bones, and some of these animal remains may resemble those of humans to hikers or lay hunters. For example, a decomposed bear paw, obtained as a souvenir that has been declawed and had its fur removed, may be mistaken for a human hand and thus brought to the attention of a forensic pathologist [4]. In these cases, being able to differentiate between human and non-human remains is pivotal. To distinguish entire bones, it is usually sufficient to base this differentiation on visual investigations by a person trained in anthropology, osteology, or anatomy. Bone fragments in turn may require microscopic analysis of osteons to distinguish between human and non-human origin. The identification of organs and soft tissue remains may be challenging and is not always visually feasible. Furthermore, it is dependent on the degree of preservation, degradation and putrefaction of the remains [4]. In the following, we present the use of an immunochromatographic rapid test (IRT) in this context. The use of these test devices for detecting human blood at crime scenes is described in the literature [5–8]. Hochmeister et al. [5] evaluated the use of IRT for the forensic identification of human blood in detail. The publication presented the Hexagon OBTI test device as a robust tool for the detection of human blood and even noted its suitability for aged and degraded material. Furthermore, the use of IRT to identify the origin of soft tissue remains was noted because of its specificity for human hemoglobin. In the following, we focus on this statement with greater detail and evaluate the use of IRT for differentiating between human and non-human soft tissue remains in the field of forensics. The respective advantages and disadvantages of this method to distinguish between human and non-human remains are demonstrated by two forensic cases. Additionally, the use of the IRT on degraded samples of several strongly decomposed bodies is evaluated. For the further validation of the IRT method, several assays of various animals were performed to detect cross-reactions. The aim of this study is to validate the IRT as a simple method for distinguishing between human and non-human soft tissue remains at the site where such remains are found.

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2. Materials and methods

2.1. Forensic relevant cases

2.1.1. Case 1

The soft tissue remains were internal organs (liver, stomach and bowel). The remains were found by a water-guard at the lake. A veterinary pathologist could not definitively exclude the possibility that the remains were human by visual examination (Fig. 1). Therefore, a forensic identification procedure was required. Due to the degree of decomposition, microscopic analyses were not valid.

2.1.2. Case 2

A 15-year-old female was hit by a train while crossing railway tracks at night. Some fragments and soft tissue remains of the dead body were collected by the police. To determine the identity and considering the young age, the body was brought to the institute of forensic medicine. Unexpectedly, an additional second liver was commingled with the body. Thus, further examinations were required to determine whether the second liver was of human origin. The deceased underwent postmortem computed tomography (PMCT) (Fig. 2). Whole-body PMCT examination was performed using a 128-slice scanner (Somatom Definition Flash, Siemens Medical Solutions, Forchheim, Germany).

2.2. Immunochromatographic rapid test and mtDNA-based species identification

An ordinary IRT (Hexagon OBTI test device, Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany) for the detection of fecal occult blood was used to distinguish between human and non-human origin. The Hexagon OBTI test consists of two components: a collection tube with a TRIS buffer and the applicator stick plus the test device. To detect hemoglobin, the applicator stick was held onto the sample. In cases 1 and 2, the measurements were performed in the depth of the liver by incising it with a clean cut. After reinserting the stick in the tube, the tube was shaken. In the next step, the tip of the tube's cap was broken off, and two drops were dripped into the opening of the test device. The test principle can be summarized as follows: if it is present, human hemoglobin reacts with a reagent consisting of colored particles and monoclonal anti-human hemoglobin antibodies. This immunocomplex migrates to the testing zone. The appearance of a test line (T-line), which indicates a positive result, occurs when the immunocomplex is captured by an immobilized additional antibody against human hemoglobin. Unreacted reagents migrate further and are trussed in the control line (C-line) by immobilized anti-mouse antibodies. Correct function and correct handling are indicated by the C-line (Fig. 3) [9].

In addition, an mtDNA-based species identification method was performed on cases 1 and 2 to identify the animal species. The mtDNA-based species identification was described by Morf et al. for identifying bushmeat species in wildlife forensics [10].

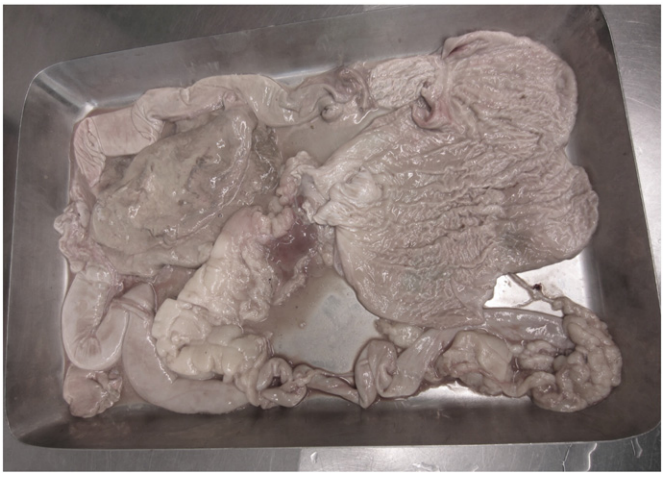

Fig. 1. The remains could not be conclusively identified as non-human by a veterinary pathologist. The remains showed a greater degree of putrefaction.

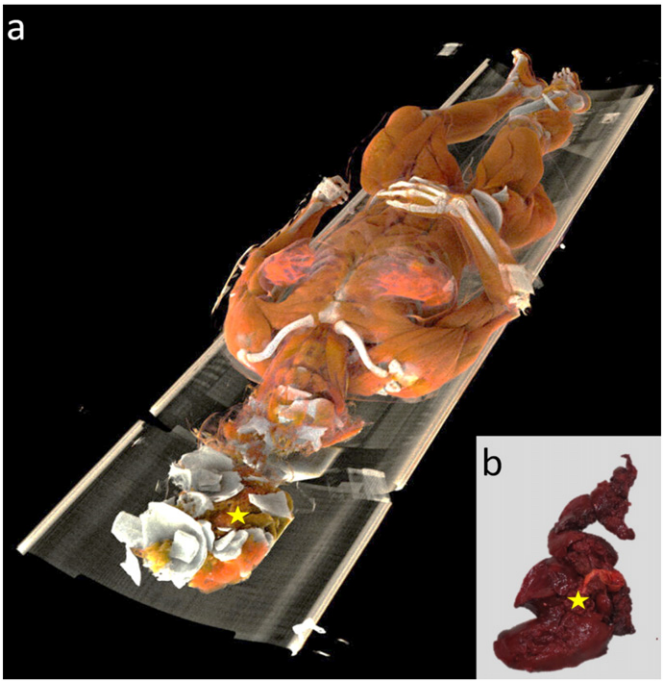

Fig. 2. Volume rendering of the postmortem computed tomography of the deceased (a). An additional second liver (b) was commingled with the remains (yellow asterisks).

2.3. Decomposed samples study

Considering the possibility of a lack of hemoglobin in the decomposed tissue samples causing false negative results, the IRT was used on several samples of decomposed bodies. The decomposed samples study involves analyzing the decomposed tissue of 20 decomposed bodies. Therefore, the applicator stick was held on the sampling point of tissue on the big toe or the ball of the foot, inserted into the sampling point of muscle tissue on the thigh, or held on the removed tissue itself, which were used for DNA identification. Additionally, the IRT was used during the autopsy of a strongly decomposed body that was found in the woods by holding the applicator stick in the sparse, liquid organ remains.

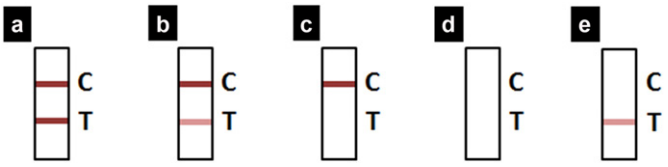

Fig. 3. Positive result: The appearance of the C-line and T-line (a) indicates a positive result for human hemoglobin. A positive result is also indicated by a weak T-line (b). Negative result: A negative result is indicated if only the C-line (c) appears. Invalid result: No appearance of any line (d) or the appearance of only the T-line (e) implies incorrect function or incorrect handling.