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SHORT COMMUNICATION

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Ninhydrin treatment as a screening method for the suitability of swabs taken from contact stains for DNA analysis

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Abstract More and more swabs containing unknown traces of biological material are submitted for forensic DNA analysis. Most of the samples are swabs taken from handled items such as tools, weapons and handles etc. Therefore, we tried to develop a screening method in order to focus the investigation on samples containing biomolecules, such as amino acids which might be associated with nucleic acids. A total of 285 swabs taken from various items collected during crime scene investigations were treated with ninhydrin which leads to a purple colour for samples containing amino acids. Of the swabs 158 were classified as ninhydrin positive and 76% of these samples yielded DNA profiles that fulfil the criteria for inclusion in the German national DNA database (profile frequency greater than 1 in 100,000) or in DNA mixtures which could at least be compared with suspects. In comparison only 9% of the 127 samples shown to be ninhydrin negative, revealed a usable DNA profile. Consequently, ninhydrin treatment was found to be an effective screening method which resulted in an increase in the rate of successfully typed samples and subsequently in a reduction of the costs due to the lower number of samples that needed to be typed.

Keywords Ninhydrin · DNA typing · Contact stains

Introduction

In the last few years the progress in DNA profiling methods together with the increasing sensitivity of the DNA marker systems, has led to the possibility of detecting very low levels of DNA. Low copy number (LCN) DNA profiling methods enable the successful investigation of samples which have simply been touched (Whitaker et al. 2001; Gill 2002; Rutty 2002; Rutty et al. 2003). Subsequently more and more stains which show no visible source of biological material were collected from crime scenes. For many crime scene officers, suspected contact between the offender and an item justifies swabbing and these samples are submitted to forensic DNA laboratories for investigation. However, whether every single sample contains biological material including DNA is unknown. In many cases this policy results in a high number of swabs being taken from one crime scene. Because of the costs of DNA analyses, in cases of less serious crime, we are sometimes requested to limit our investigations to a randomly chosen subset of the collected swabs. Therefore, we tried to develop a suitable screening test using ninhydrin (2,2dihydroxyindane-1,3-dione), already known as a chemical reagent for the detection of latent fingerprints on porous surfaces such as paper (Oden and Hofsten 1954). Ninhydrin reacts with amino acids resulting in a purple staining. It has been shown that ninhydrin has no influence on the quality of the DNA or the results of the DNA typing up to 56 h after treatment and dry storage (Stein et al. 1996; Fregeau et al. 2000; Grubwieser et al. 2003). In this study 285 swabs taken at crime scenes from contact stains with unknown content of biological material were stained with ninhydrin and DNA profiling was carried out (Junge et al. 2003).

Furthermore, it was examined whether ninhydrin is also suitable for the detection of non-visible biomolecules including amino acids on the surface of larger objects.

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Materials and methods

Sampling and ninhydrin treatment. A total of 285 swabs moistened with ethanol, were collected from various tools, weapons, handles etc. during crime scene investigations. After drying the swabs for 6 h at 42°C they were sprayed with the ninhydrin-containing reagent NIN-Print SB0045 (Stöckle, Gräfelfing, Germany). During

further incubation steps the purple colour developed at 42°C for 12 h and then for up to 3 days at room temperature. To avoid contamination the swabs were packed into paper envelopes after the second 42°C incubation step. All incubation steps were carried out in the dark. In a series of control experiments unused swabs from different manufacturers were treated in the same way.

DNA extraction and purification. From all swabs showing even a slight purple colour DNA extraction was carried out using a 5% Chelex solution including proteinase K (Walsh et al. 1991) in a total volume of 500 µl. Furthermore 127 samples shown to be ninhydrin negative (i.e. showing no change in colour) were tested in parallel as a control group. The chelex extracts were further purified and concentrated with Microcon 100 tubes (Millipore Corporation, Bedford, MA) to a final volume of 50 µl. In every experiment a negative control was extracted together with the real samples.

PCR and electrophoresis conditions. A multiplex PCR was performed using the genRES MPX-2 amplification kit (Serac, Bad Homburg, Germany) which allows simultaneous amplification of the vWA, ACTBP2, TH01, D21S11, D8S1179, D3S1358, FGA and D18S51 loci plus the sex-determining amelogenin locus corresponding to the 8 STRs included in the German national DNA database. For each sample 2 DNA concentrations (4 and 18 µl per sample in a total reaction volume of 25 µl) were used for amplification in a PE 9600 thermal cycler according to the manufacturer's instructions using a 32 cycle programme. The PCR products were analysed on the ABI PRISM 310 genetic analyser (Applied Biosystems, PE Corporation, Foster City, CA).

Example of the direct application of ninhydrin on a stain. A cotton bag was left behind at the crime scene by the offender. Investigations on swabs taken from the handles of the bag revealed no usable DNA profile. In order to search for any other biological material from the offender, ninhydrin was sprayed onto the bag and several dark purple spots could be detected (Fig 1), whereas the handles showed only a weak staining. From one of the coloured spots and the handles, samples were taken and treated as described.

Results

From the various brands of unused swabs tested as a negative control group only one type (swabs with a wooden stick) showed a light purple shading. For all other brands no change in colour could be observed. Therefore all further investigations were carried out with one kind of plastic stick swabs that showed no false positive reactions (Kokett Wattestäbchen, Pantos Produktions, Buchholz, Germany).

Of the 285 samples treated with NIN-Print, 158 developed a purple colour, ranging from deep purple to even a very light purple shading (all rated as ninhydrin positive). For the remaining 127 swabs no change in colour was observed. Of the 158 ninhydrin positive swabs, 120 (76%) yielded DNA profiles either suitable for inclusion in the German national DNA database (complete profiles with results in all 8 systems or partial profiles with frequencies greater than 1 in 100,000) or DNA mixtures which could be compared with suspects. In contrast, only 11 of the negative samples (9%) revealed a DNA profile suitable for inclusion in the DNA database. To exclude contamination, all profiles have been compared to the staff members of the laboratory and no matches were found.

A direct relationship between the intensity of the purple colour after ninhydrin treatment and the quality of the resulting DNA profile could not be found. In rare cases even lightly stained swabs yielded complete profiles, whereas some very dark purple coloured samples showed only weak or no signals at all.

The investigation of the coloured spot taken from the cotton bag revealed alleles for all 8 STR-marker systems stored in the national database, resulting in a full profile with a frequency of 1 in a billion.

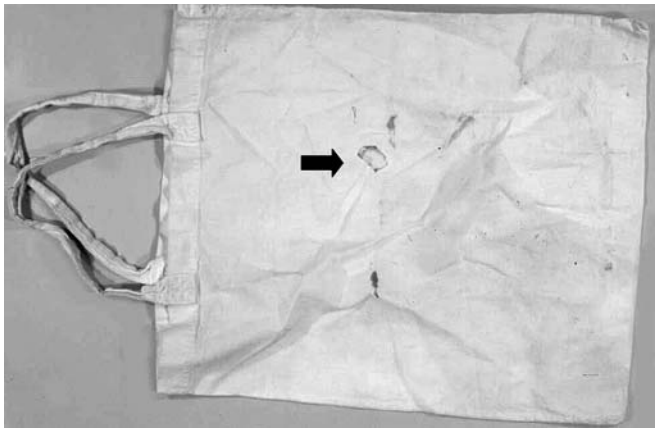

Fig. 1 Cotton bag showing several dark purple spots after ninhydrin treatment. The *arrow* indicates the localisation of the stained area that was cut out and typed

Discussion

Ninhydrin reacts with the amino acids which can be found in biological stains, thus only indirect evidence for the presence of DNA is obtained. Although there is no direct correlation between the intensity of the purple colour and the quality of the DNA profile, the investigation revealed that treatment with ninhydrin is well suited for the screening of swabs with unknown content of biological material. By limiting the investigation to ninhydrin positive samples and consequently samples containing detectable biological material, it was possible to increase the rate of successfully typed samples and to reduce the costs for the police units for further DNA investigation.

However, a minor fraction of the negative swabs gave results in the DNA investigation. Therefore, this screening method should be applied to less serious cases in which not all swabs are submitted for a DNA investigation.

Depending on the case and considering the fact that ninhydrin is a marker for amino acids and not for DNA, the investigation of the negative swabs should be taken into account in serious crime cases. However, in order to avoid false positive results it is indispensable to test unused control swabs before applying the ninhydrin screening method.

The example with the cotton bag demonstrated that treatment with ninhydrin also seems to be an effective method for the detection of cellular material on handled items.

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