FORENSIC-LIKE STAINS ON DENIM: REMOVAL OF AN RT-PCR INHIBITOR FROM RNA SAMPLES ISOLATED FROM BLUE JEAN MATERIAL

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Although serological methods are widely used to determine the source of biological stains, in some cases the results are presumptive. mRNA profiling of forensic stains has emerged as a possible means of providing more information from the evidence. Based on the fact that each cell type must express different mRNA's to carry out their specific role, mRNA profiling can identify the source of a forensic stain as menstrual blood versus systemic blood, saliva, semen, vaginal secretions and so on. Quantitative PCR of the housekeeping gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), expressed in all of the stain types, has been used as a means to quantify the RNA isolated from forensic-like stains (a body fluid applied to a swab or piece of fabric). Quantitative PCR is also utilized to amplify body fluid specific mRNAs. RNA yields from stains can be low, especially when dealing with stains of a very small size. For this reason, it is important to maximize the amplification of mRNAs in the sample, allowing one to detect the smallest amount possible. Denim stains, in particular, presented a challenge because they yielded lower amplification than expected when directly compared to RNA isolated from stains on white 100% cotton. It was hypothesized that PCR inhibitors were present in the denim RNA isolates. To address this question, a study involving 45 samples of semen, saliva and blood stains on indigo blue jean material and white 100% cotton sheeting was undertaken. Half of the RNA extracted from stains on denim was subjected to an additional Microcon® YM 3 centrifugal filtration clean-up step while the other half was not. When the amount of total RNA in the samples was normalized, GAPDH quantitative PCR revealed that, in general, RNA from denim stains which did not receive a Microcon® clean-up step did not amplify as well as RNA from denim stains that did.