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A GEFI collaborative exercise on DNA/RNA co-analysis and mRNA profiling interpretation

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ABSTRACT

A collaborative exercise on DNA/RNA co-analysis and RNA cell typing involving 15 GEFI (Italian working group of ISFG) laboratories was organized in collaboration with the Netherlands Forensic Institute.

Participants received: 1) PCR primers for a 19-plex mRNA profiling assay, with reference purified PCR products for each cell type targeted in the multiplex; 2) detailed protocols for DNA/RNA co-extraction, mRNA profiling, and interpretation of results; 3) a set of 8 mock forensic stains (7 single source, one a mixture of two body fluids).

All but one laboratory generated correct DNA typing results. As expected, stochastic effects were seen for low template DNA extracted from a skin stain.

As for mRNA profiling, the percentage of laboratories that correctly identified body fluids was ≥60% for blood, saliva, vaginal mucosa, semen, and skin. Success rates were < 50% for menstrual secretions, nasal mucosa, and the mixed stain (menstrual secretions and saliva).

1. Introduction

DNA/RNA co-extraction enables the single pipeline analysis of both STRs and mRNA profiles to identify body fluids [1]. To promote expertise in DNA/RNA co-analysis and interpretation of mRNA profiling data among GEFI (Italian working group of ISFG) laboratories, a collaborative study was organized with the support of the Netherlands Forensic Institute.

2. Materials and methods

Participants were provided with: 1) PCR primers for a 19-plex mRNA profiling assay [2] and reference purified PCR products for each cell type targeted in the multiplex; 2) detailed protocols for DNA/RNA co-extraction [3], mRNA profiling, and interpretation ("scoring") of results [4]; 3) a set of 8 mock forensic stains (QC1-QC8).

Participants were free to adopt the quantitation method and STR panel of choice for DNA analysis of stains. The following STR kits or combination of kits were used: Identifiler Plus (n = 3), NGM (n = 2), Globalfiler (n = 2) (Thermo Fisher Scientific, Waltham, MA); PowerPlex ESX 17 (n = 3), Fusion (n = 1) (Promega, Madison, WI); ESSplex SE Plus (n = 2) (Qiagen, Hilden, Germany); Identifiler Plus and PowerPlex ESX 17 (n = 1).

3. Results

Fifteen GEFI laboratories participated in the study. Results from one laboratory, which experienced problems in the co-extraction of DNA/RNA, were excluded from data analysis. Remaining participants provided correct DNA profiling results for all stains excluding QC7 (skin). Because of the limited amount of DNA isolated from QC7 (reported average DNA concentration 0.036 ng/μl), 4 laboratories could not generate any STR profile, and stochastic effects were frequently seen in STR typing results of other participants.

Results of 19-plex mRNA profiling experiments are shown in Fig. 1. Differential amplification was evident in several body fluids, with weak signals observed for CD93 in blood, MYOZ1 in vaginal mucosa, BPIFA1 in nasal mucosa, and LCE1C in skin. CDSN was expressed in most body

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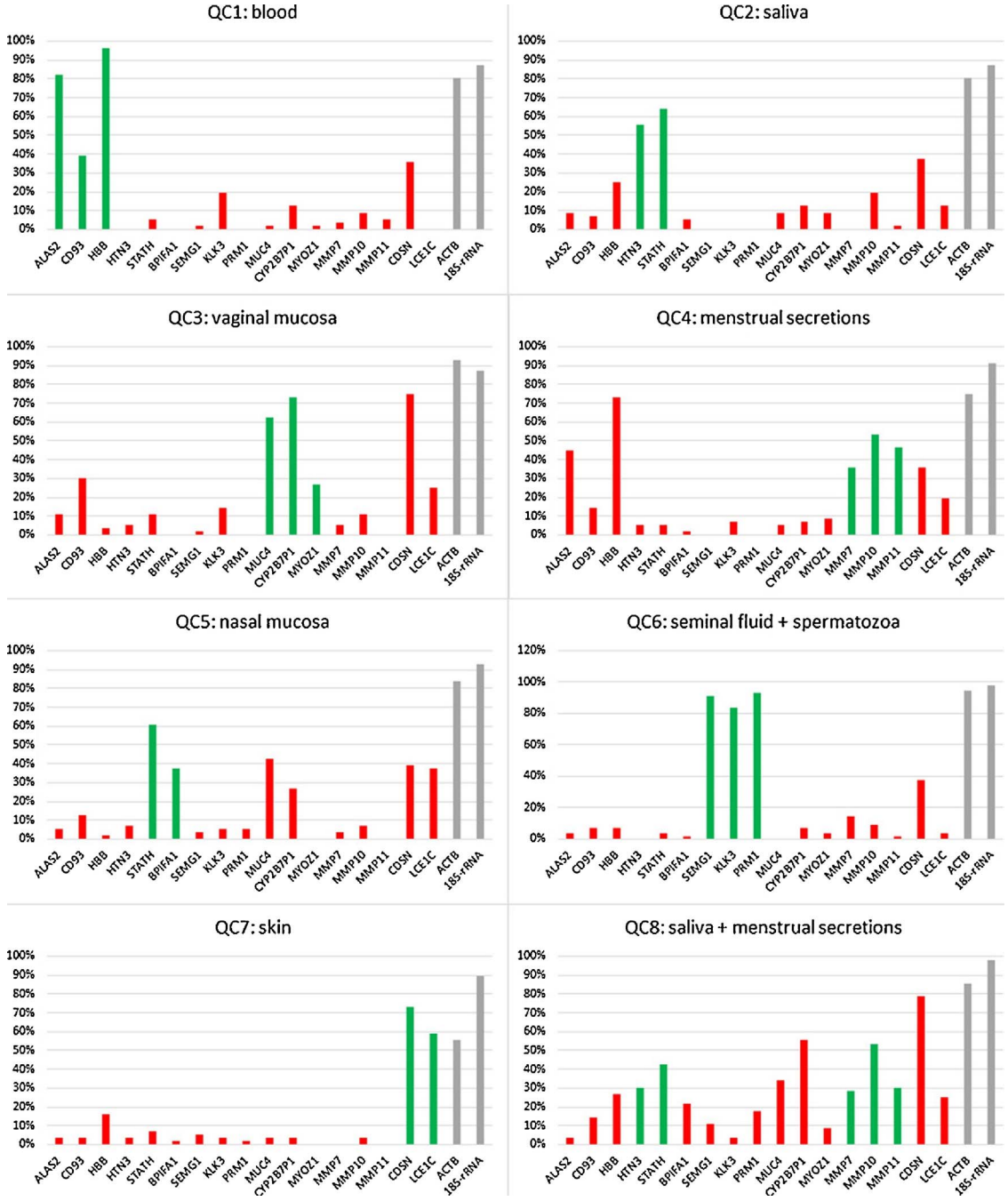

Fig. 1. Detection frequency of tissue-specific and housekeeping markers in mRNA profiling experiments (four replicates of the multiplex PCR reaction were performed for each stain). Stain-specific markers are highlighted in green, markers expected to be co-expressed in light green, housekeeping genes in grey, other markers in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fluids. Although cross-reactivity could not be excluded in collection sites of QC3, QC4 and QC8 (vulval vestibule) and QC5 (rim of the nostrils), the fact that the unexpected observation of CDSN was recurring in the same 5 laboratories also suggested possible contamination of plastics/reagents. On the contrary, CDSN expression was observed by all but one GEFI laboratory in vaginal mucosa, confirming the occurrence of CDSN false positive signals in such tissue [5].

Scoring results are summarized in Fig. 2. Most of the participants successfully identified blood, saliva, vaginal mucosa, semen, and skin. Correct classification of menstrual secretions, nasal mucosa, and mixed stain QC8 was more problematic (< 50%).

4. Discussion

Superior results of mRNA profiling in skin stain QC7, compared to STR typing, may be partly explained by CDSN artefacts observed in 5 laboratories. Nevertheless, one of the laboratories that correctly identified QC7, without evidence of CDSN aspecific signals in other tissues, was among those unable to provide STR typing results for QC7. This is consistent with previous observations showing that skin mRNA markers may remain detectable even when DNA profiling efficiency decreases [2].

Susceptibility to drop out of CD93 and BPIFA1 was also confirmed [2]. CD93 is a leukocyte marker, which has been shown to be less sensitive than erythrocyte markers ALAS2 and HBB [2]. Differential

Fig. 2. Scoring of mRNA profiling results: "observed" (O), "observed and fits" (OF), "sporadically observed" (SO), "sporadically observed and fits" (SOF), "not observed" (NO). Percentage of laboratories correctly identifying the stains is highlighted in green. Errors (sporadic or no observation of expected tissues, observation of unexpected tissue) are shown in red. Other results, like observation, or sporadic observation, of tissues fitting with the stain (*e.g.* blood in menstrual secretions), and sporadic observation of unexpected tissues are shown in grey. Co-expression of skin was considered possible in collection sites of QC3, QC4 and QC8 (vulval vestibule) and QC5 (rim of the nostrils). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

amplification of CD93, however, did not affect positive scoring of blood, thanks to the presence of three blood-specific markers in the multiplex. Drop out of BPIFA1 markedly reduced detection rates of nasal mucosa, yet the inclusion of this marker in mRNA profiling assays remains crucial, at present, to discriminate nasal mucosa from saliva and vaginal mucosa (given the frequent cross-reactivity with vaginal markers), and nosebleed from peripheral blood.

5. Conclusion

For many of the participants, this collaborative exercise represented a first exposure to DNA/RNA co-analysis. Awareness of the methodological and interpretative challenges of mRNA profiling, highlighted by the study, will be beneficial to the future implementation of this technique in GEFI laboratories.

Conflict of interest statement

None.

Role of funding

None.

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