



### **B69 Validation of the Qiagen BioRobot™ 604 for the Extraction of DNA From Buccal Swabs**

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The goals of this presentation are to compare the Qiagen BioRobot<sup>®</sup> 9604 extraction method with Chelex and Organic extraction methods on buccal swabs and to present this data to the forensic community.

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The objective was to validate the Qiagen BioRobot™ 9604 for use with Puritan cotton tip and Gibco BRL C.E.P. buccal swabs. The Qiagen BioRobot<sup>®</sup> 9604 is designed to automate routine extraction of DNA from buccal swabs. The validation involved the comparison of DNA extracts obtained from the Qiagen BioRobot<sup>®</sup> 9604 with DNA extracts obtained using a standard swab Chelex extraction method and a swab organic extraction method. Comparisons were based on quantitation results using the QuantiBlot<sup>®</sup> Human DNA Quantitation Kit and on STR analysis of PCR products using the AmpFISTR Cofiler<sup>®</sup> and the AmpFISTR Profiler Plus PCR Amplification Kits. Experiments were performed according to the Qiagen BioRobot<sup>®</sup> 9604 user's manual.

Swabs, 4 cotton type and 4 C.E.P. type, were collected from 10 individuals. The swabs were gently rubbed against the inside of the volunteer's cheek and gum line for approximately 30 seconds. For one individual an additional C.E.P. swab was collected without extensively rubbing it along the cheek and gum line. This was done to test the sensitivity of the Qiagen on a poorly collected sample. A C.E.P. and a cotton type swab was extracted for each individual for each method of extraction that was tested for a total of 60 swabs. An extra C.E.P. type swab was also extracted to test sensitivity as stated above.

The 21 swabs that were extracted using the Chelex method gave visual results on the QuantiBlot<sup>®</sup> film upon quantitation. The samples contained approximately .0625.5ng/ul of DNA. The 21 swabs that were extracted using the organic method also gave visual results on the QuantiBlot<sup>®</sup> film upon quantitation. The samples all contained greater than 2ng/ul of DNA. The 21 swabs that were extracted using the Qiagen BioRobot<sup>®</sup> 9604 contained .5-2ng/ul of DNA. The sample that was used to represent a poorly collected sample had less DNA (.06125ng/ul) than the swab collected the proper way (.25ng/ul).

Full profiles from the Chelex extracts were generated for 13 of the samples using Profiler Plus and 15 of the samples using Cofiler. All of the organic extracts gave full profiles for both kits. Full profiles were generated for both Profiler Plus and Cofiler for 17 of the swabs that were extracted using the Qiagen BioRobot. The 4 C.E.P. type samples extracted on the Qiagen BioRobot gave no profile with both Profiler Plus and Cofiler. All 4 were amplified with 10 ul of DNA and contained less than .03125ng of template DNA. The sample that was taken to represent a poorly collected specimen gave a full profile for all extractions. All of the samples gave a DNA profile that was consistent with the ones that were on file for the volunteer. No contamination was observed. The 4 samples that did not generate a profile using Qiagen gave full profiles when extracted using the Chelex and Organic methods. It was observed that the C.E.P. swabs were not completely covered by the tissue lysis buffer and pro K. This is believed to be the reason that the four C.E.P. type swabs did not yield DNA.

As a follow up to this study, it was found that sample initially extracted using the Qiagen BioRobot could be reextracted using the organic method. There was between 1 ng/ul and >2 ng/ul of DNA in the reextracted samples when quantitated with QuantiBlot<sup>®</sup>. The second extraction yielded enough DNA to get a full profile from the swabs.

The Qiagen BioRobot<sup>®</sup> 9604 has proven to be acceptable for use with both comb and cotton type Buccal Swabs. The in-house validation experiment shows that the concentrations of DNA obtained from the Qiagen extraction are better than those obtained from the standard Chelex. The Qiagen extraction did not give a DNA yield that was better than the Organic extraction method.

#### **Buccal Swabs, Qiagen BioRobot, STR**