

## Testing the Effectiveness of the PrepFiler™ Kit for DNA Extraction from Forensic Samples (An Overview of Test Site Data)

### Introduction

Optimizing the forensic sample processing workflow requires piecing together a multitude of techniques with the ultimate aim of generating high quality data for use in forensic investigations. Over the last 12 years, Applied Biosystems has devoted significant effort to the development of highly optimized DNA quantitation and STR amplification chemistries that, combined with efficient capillary electrophoresis platforms, now form the backbone of many forensic DNA laboratories worldwide. The newest addition to this workflow is the PrepFiler™ Forensic DNA Extraction Kit, which has been designed specifically to improve the overall yield and quality of DNA isolated from forensic samples and thus maximize the potential of all the downstream processes.

### Putting the PrepFiler™ Kit to the Test

As part of the validation process, Applied Biosystems tested the performance of the PrepFiler™ kit against a standard organic Phenol/Chloroform process and 3 other commercially available chemistries on a variety of forensic-type samples. The extracts were evaluated for DNA concentration, total quantity of DNA isolated, and purity using the Quantifiler® Human DNA Quantification Kit. An example of the results is shown in Figure 1.

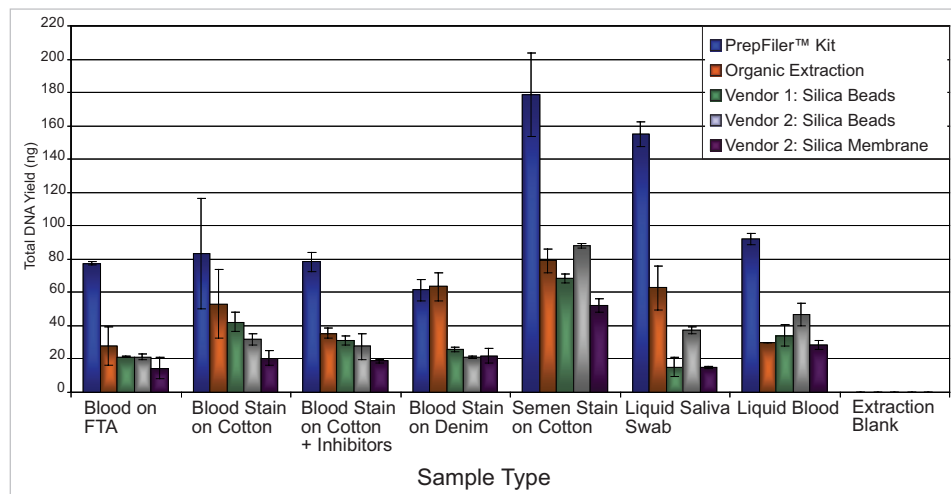
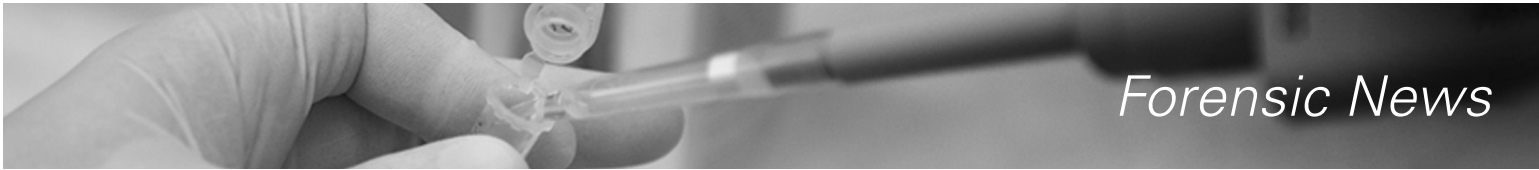


Figure 1: Comparison of the total yield of DNA isolated from simulated forensic casework samples between the PrepFiler™ kit and 4 other commonly used methods.

In all cases, the PrepFiler™ kit matched or exceeded the concentration and total yield of DNA produced by other methods. For certain sample types, the PrepFiler™ kit yielded greater than 100% more DNA than the other technologies tested. Increasing the yield of DNA isolated provides the forensic scientist with more options in terms of the types and numbers of analyses that can be performed on the sample.



### Test Site Results for the PrepFiler™ Kit

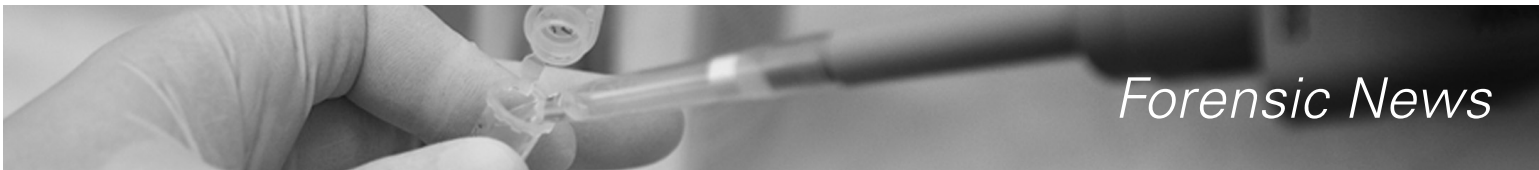
Testing simulated forensic-type samples in an Applied Biosystems laboratory is an important part of the development and validation process, but to more effectively challenge and evaluate the capabilities of the PrepFiler™ kit, we rely on the cooperation of our crime laboratory test sites to test the chemistry on the same simulated forensic-type samples as well as a wide variety of non-probative samples or samples from adjudicated cases.

To ensure a valid comparison could be made between results obtained from the different testing laboratories, we supplied a standard set of samples (see Table 1) for each laboratory to extract using the PrepFiler™ kit and their own routine extraction method. In addition, we asked them to extract some of their own, actual casework samples.

Sample Name	Sample Description
BFTA-1	Blood stain on FTA paper (2µl)
BFTA-2	Blood stain on FTA paper (2µl)
BSC-1	Blood stain on non-coloured cotton fabric
BSC-2	Blood stain on non-coloured cotton fabric
BSCI-1	Blood treated with inhibitor on cotton fabric
BSCI-2	Blood treated with inhibitor on cotton fabric
BSD-1	Blood on blue denim (2µl)
BSD-2	Blood on blue denim (2µl)
SSC-1	Semen on non-coloured cotton fabric (1µl)
SSC-2	Semen on non-coloured cotton fabric (1µl)
SalSw-1	Saliva on cotton swab (50µl)
SalSw-2	Saliva on cotton swab (50µl)

Table 1: Details of the 12 samples provided to each test site crime laboratory for testing. All samples were extracted using the PrepFiler™ kit and the standard method in use at each test site.

Each test site compared the PrepFiler™ kit to a different, commonly used extraction method to provide an indication of the level of performance in comparison to other methods and the level of consistency of performance between the sites with multiple operators in different environments. Some of the results are shown in Figures 2, 3 and 4.



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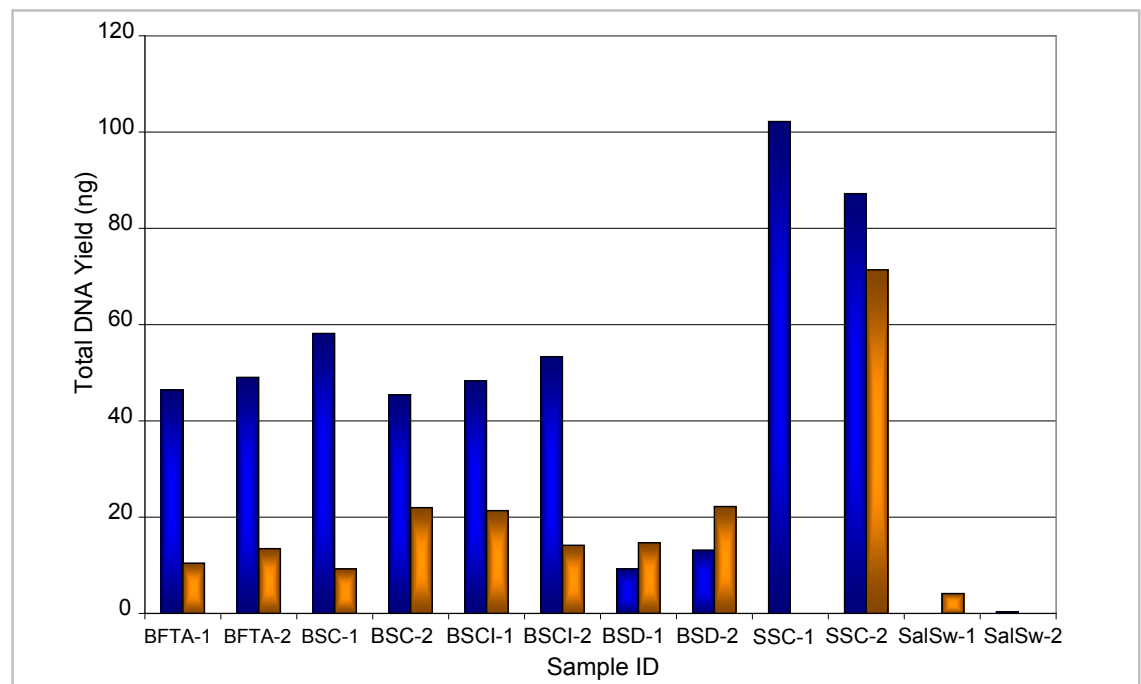
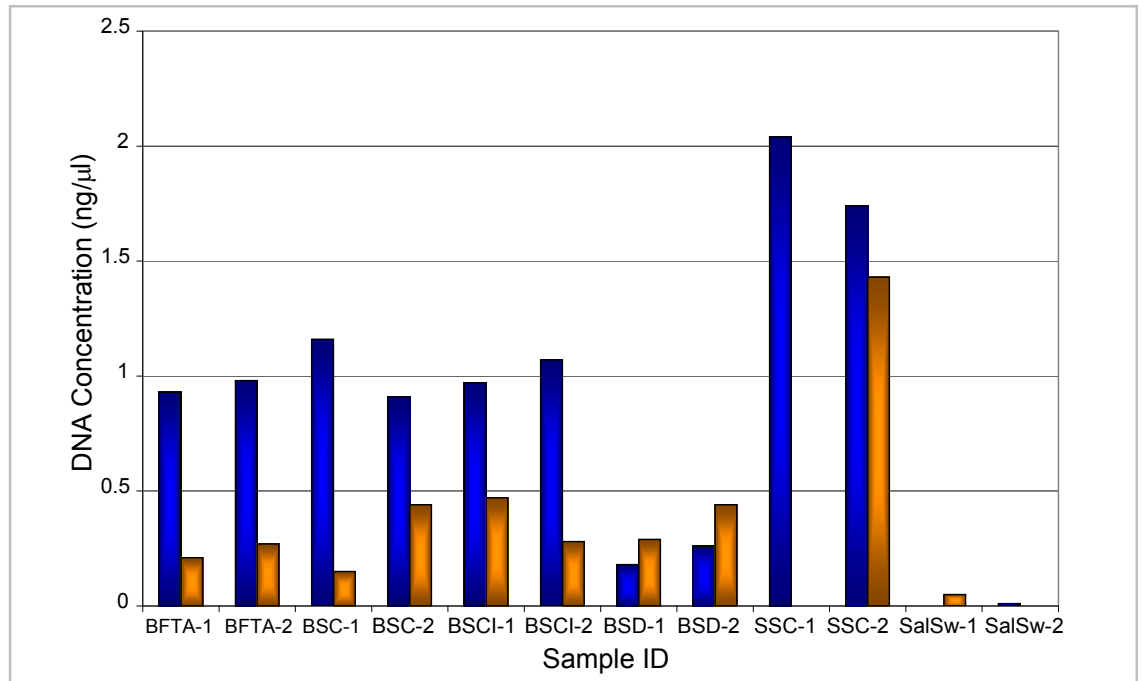
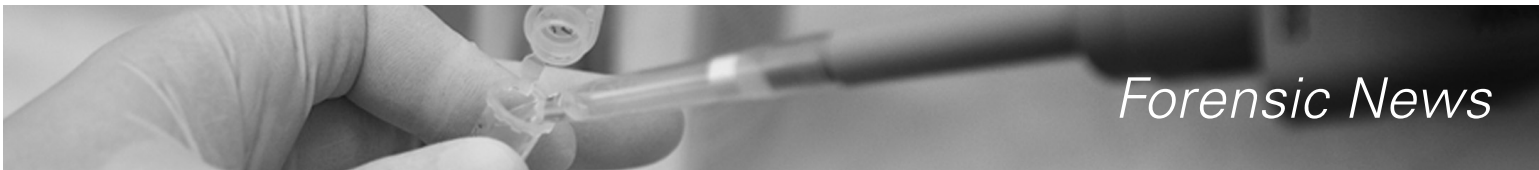


Figure 2: Comparison of the DNA concentration of extracts (top) and total DNA yield (bottom) obtained using the PrepFiler™ kit (blue bars) and an organic extraction method (orange bars) by Test Site "S".



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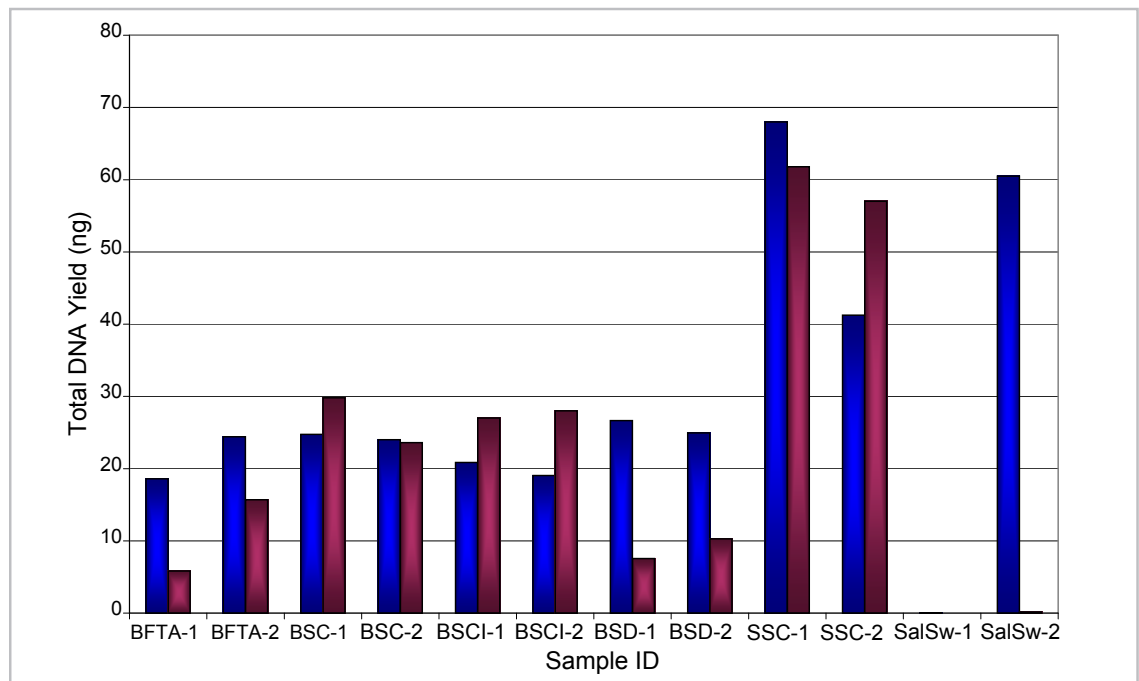
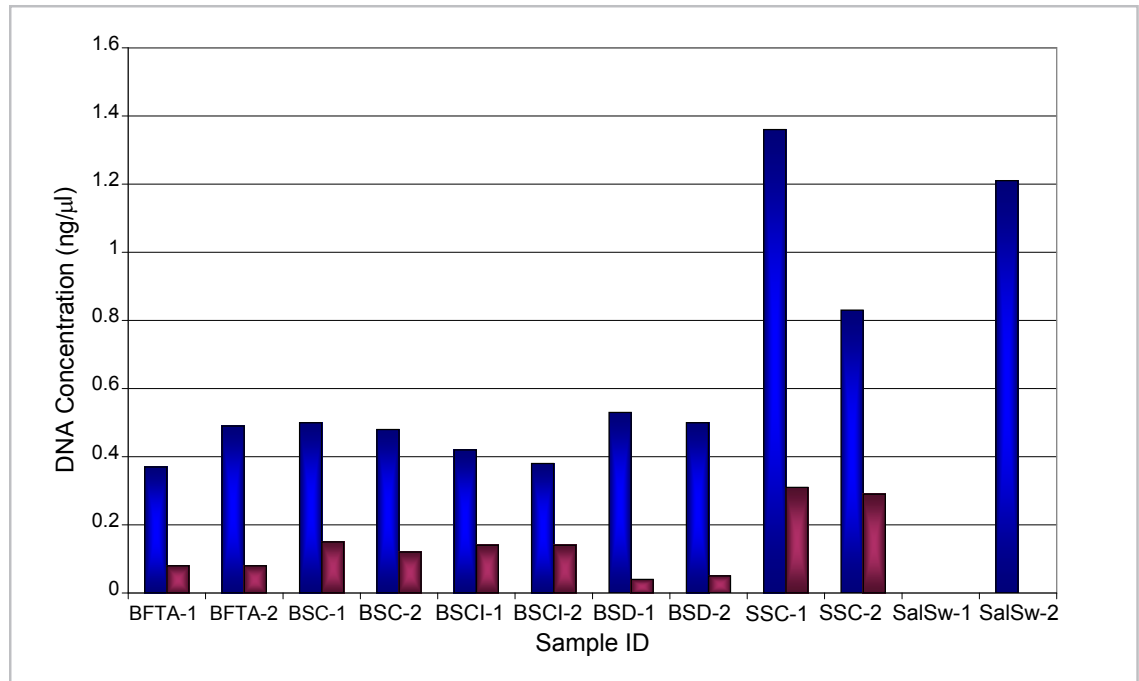
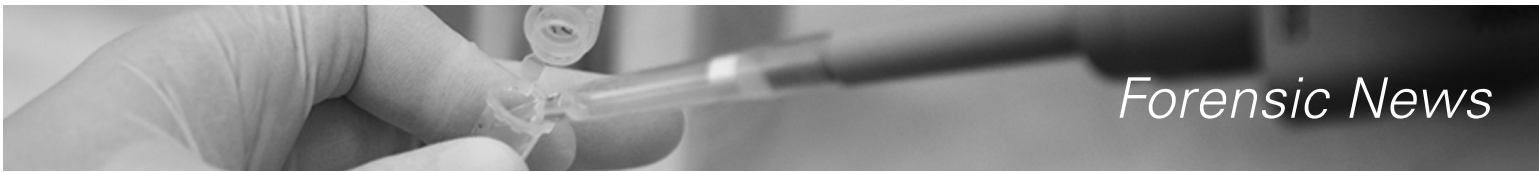


Figure 3: Comparison of the DNA concentration of extracts (top) and total DNA yield (bottom) obtained using the PrepFiler™ kit (blue bars) and a silica membrane method (dark red bars). Data courtesy of David Young, Texas Department of Public Safety – Lubbock.



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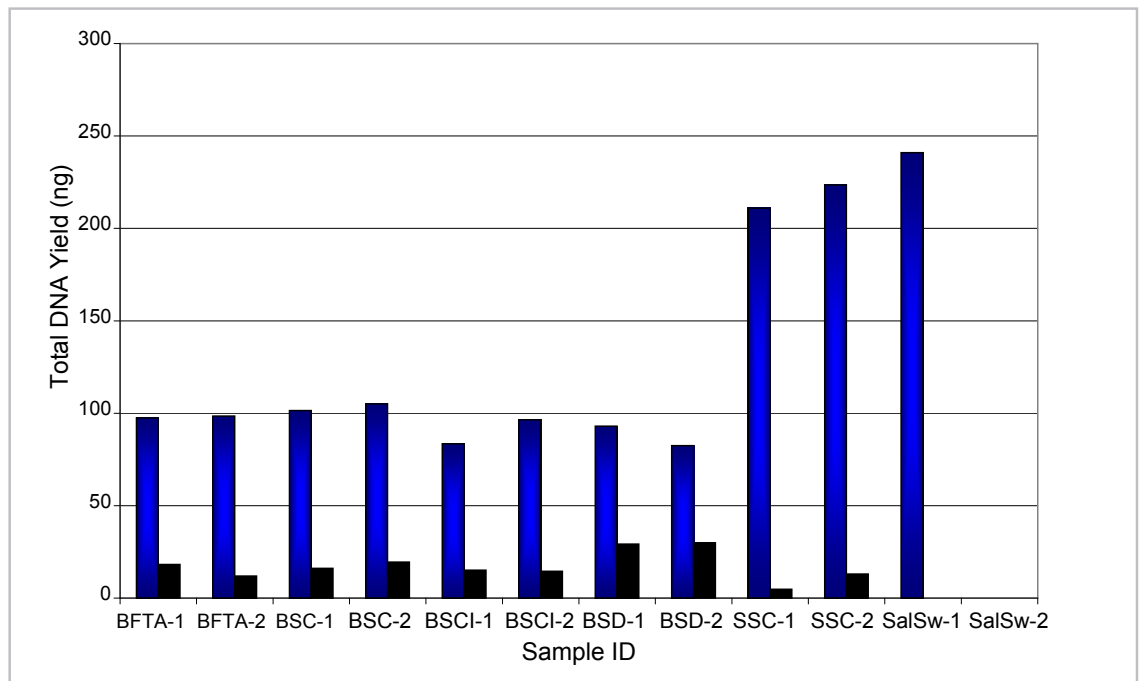
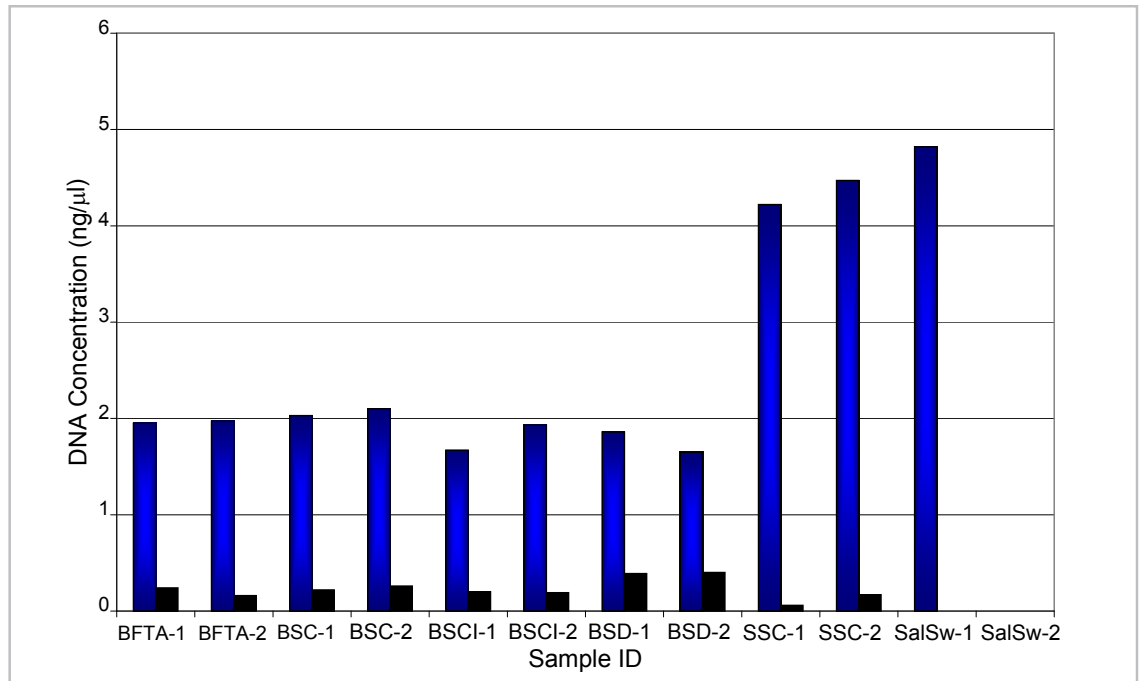


Figure 4: Comparison of the DNA concentration of extracts (top) and the total DNA yield (bottom) obtained using the PrepFile™ kit (blue bars) and an ion exchange extraction method (black bars). Data courtesy of Dr. Naseem Malik, University of Bern – Institute of Legal Medicine.



For almost every sample tested across all of the test sites, the PrepFiler™ kit delivered significantly better performance in terms of DNA concentration of the extract and total DNA yield. In the remainder of cases, the performance of the PrepFiler™ kit matched the level of performance of the other methods used by the test sites. Perhaps the most striking performance comparison results were obtained from the University of Bern. For all samples tested, the PrepFiler™ kit outperformed the ion exchange method both in terms of DNA concentration and total DNA yield by at least 3-fold.

Taking the test site data as a whole, two samples in particular help to illustrate the increased level of performance delivered by the PrepFiler™ kit. Sample SalSw-2 (50 µl liquid saliva on a swab, donor 2) failed to yield any DNA when extracted using a silica membrane-based extraction but produced over 60ng total DNA using the PrepFiler™ kit. Similarly, sample SalSw-1 (50 µl liquid saliva on a swab, donor 1) failed to yield quantifiable DNA when extracted using the ion exchange method but the PrepFiler™ kit was able to produce more than 200 ng of DNA from the same sample. For both of these samples, the quantitation results were confirmed when the samples were amplified using the Identifiler® kit as shown in Figures 5 and 6.

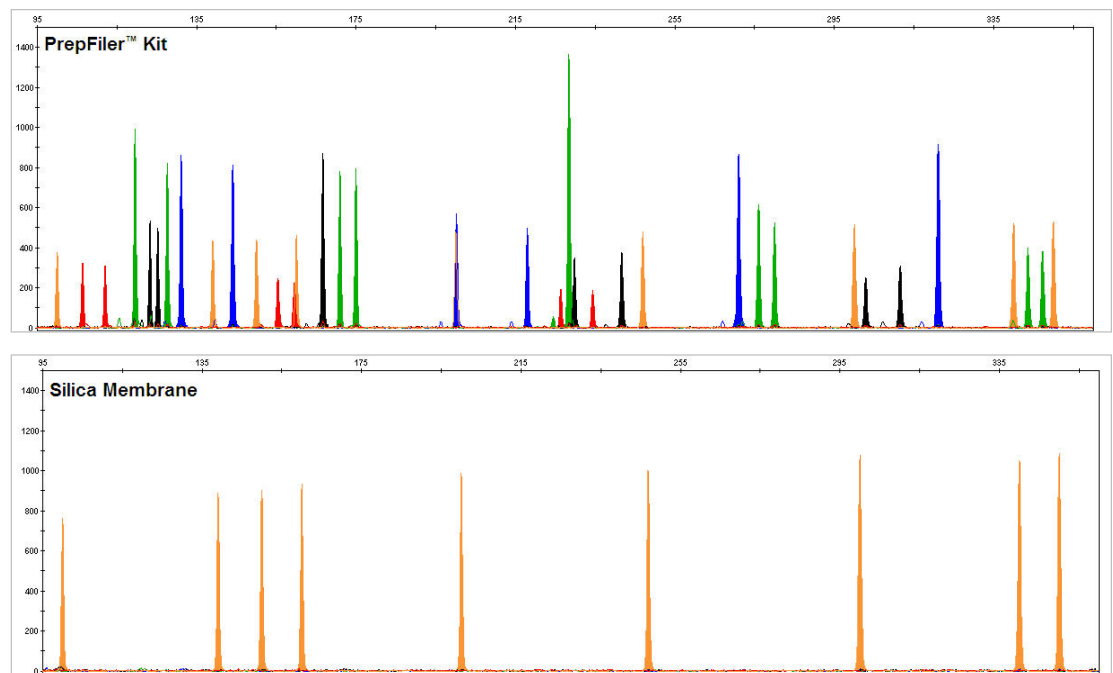
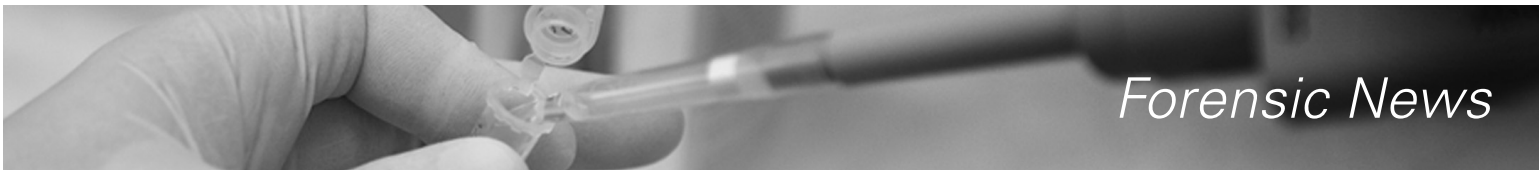


Figure 5: Identifiler® kit profiles of sample SalSw-2 extracted using the PrepFiler™ kit (top) and a silica membrane-based method. The silica-based extraction yielded no quantifiable DNA and no profile was detected as compared with the PrepFiler™ extract which produced over 60 ng of total DNA and a full and balanced STR profile. Data courtesy of David Young, Texas Department of Public Safety – Lubbock



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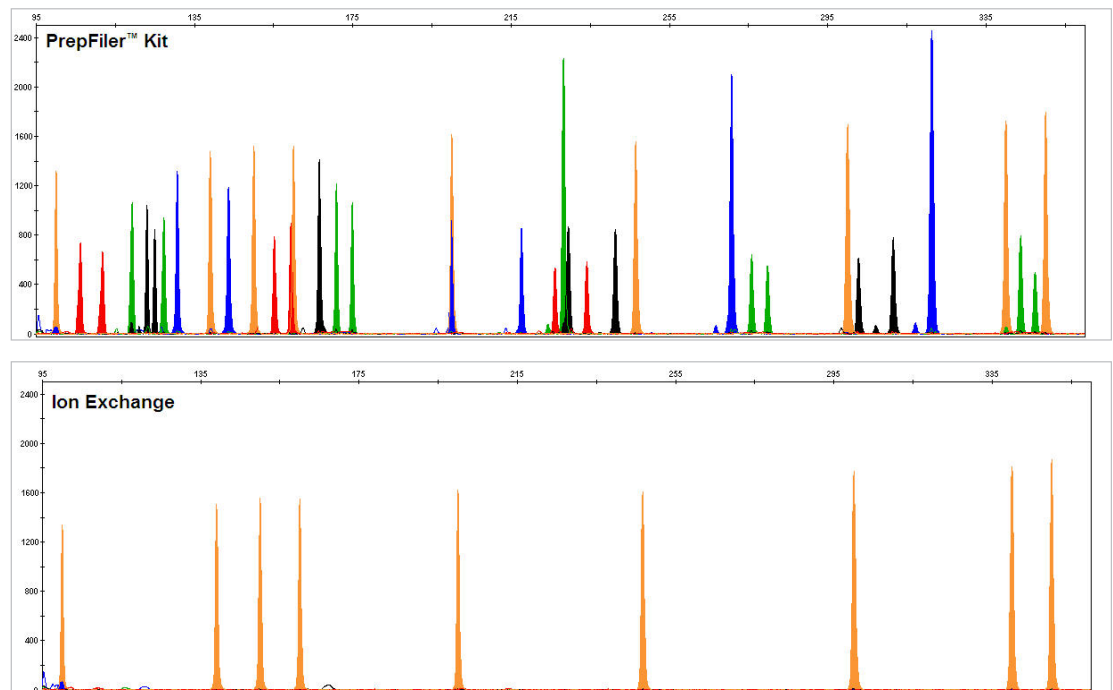
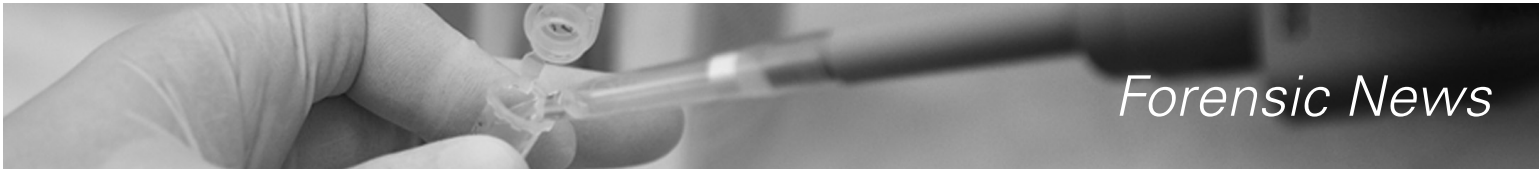


Figure 6: Identifiler® kit profiles of sample SalSw-1 extracted using the PrepFiler™ kit (top) and an ion exchange extraction method. The ion exchange extraction yielded no quantifiable DNA and no profile was detected as compared with the PrepFiler™ extract which produced over 200 ng of total DNA and a full STR profile. Data courtesy of Dr. Naseem Malik, University of Bern – Institute of Legal Medicine.

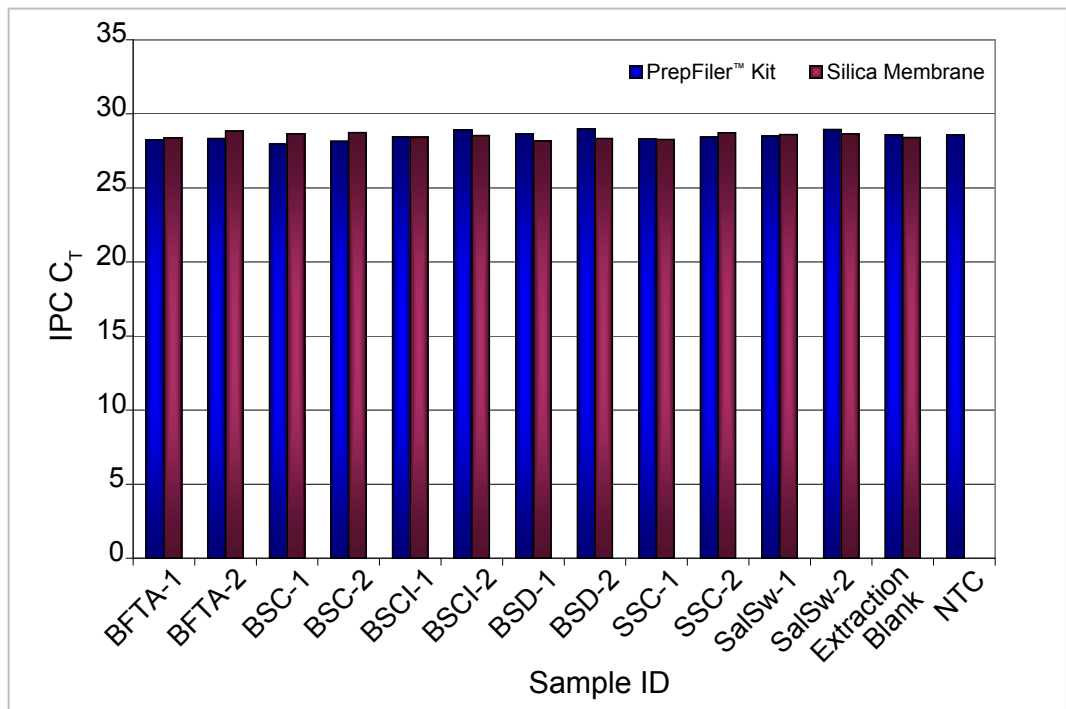
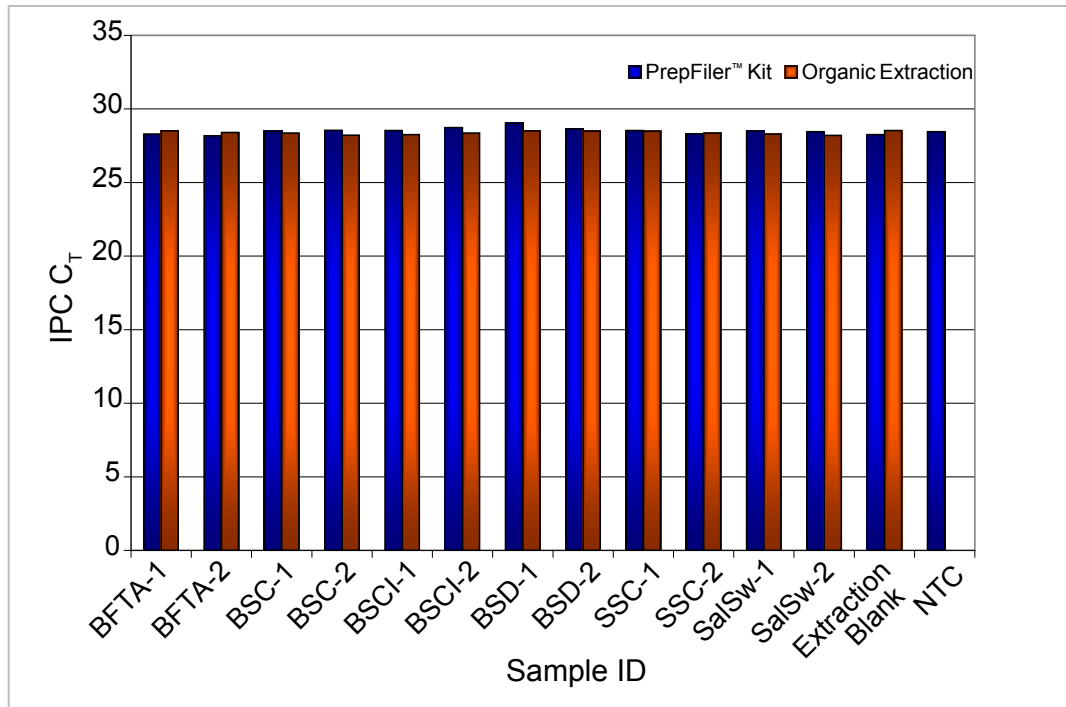
All DNA extracts generated using each of the different extraction methods tested by our collaborators were assessed for purity by comparing the  $C_T$  values generated for the samples against the negative controls. The presence of inhibitors is indicated if the sample IPC  $C_T$  exceeds the value of the IPC  $C_T$  from the negative controls by more than 1 unit (+ or -). The PrepFiler™ kit showed equivalent or better performance than the other extraction chemistries for all of the samples including those spiked specifically with mixes of inhibitors as shown in Figure 7.



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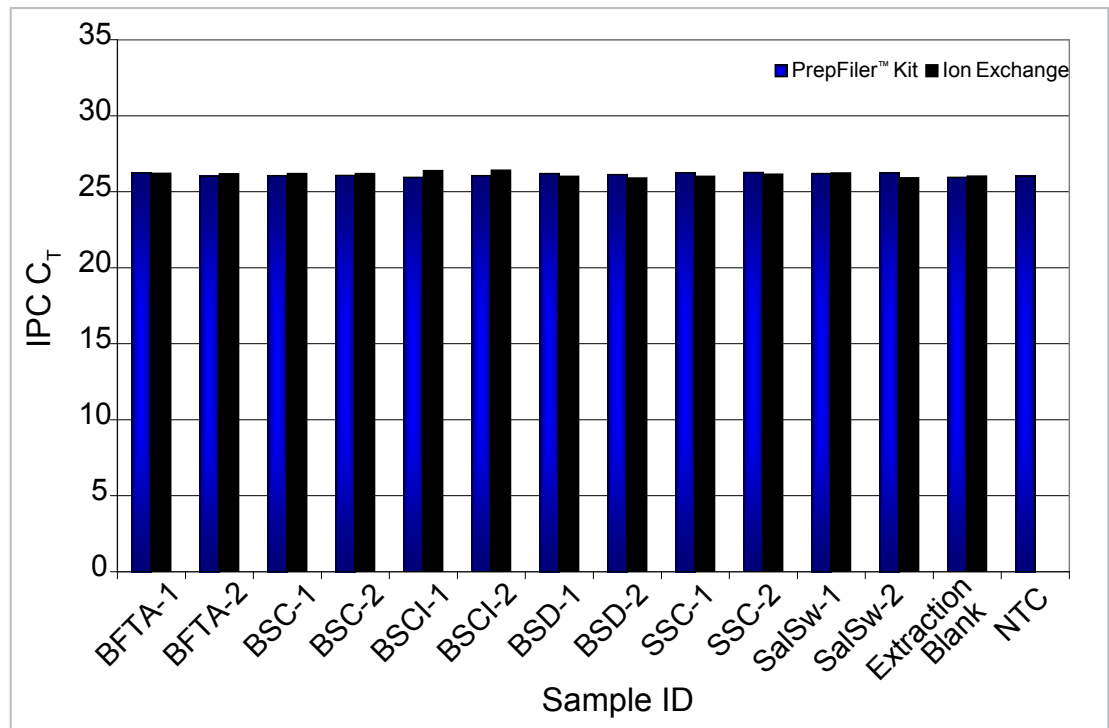


Figure 7: Comparison of the IPC  $C_T$  values generated for each sample tested using the PrepFiler™ kit (blue bars) and the in-house extraction methods (indicated on each chart) by each of the test sites. Each chart also indicates the IPC  $C_T$  value obtained for the negative controls (NTC) for comparison to the samples.

### The Verdict

The results obtained from our test site collaborators, some of which have been described here, show that while multiple extraction techniques can produce DNA of sufficient purity for STR amplification, the PrepFiler™ kit has the added advantage of delivering higher concentration and higher total DNA yields across a diverse range of sample types. The PrepFiler™ kit showed consistent levels of performance between the test sites, demonstrating a robustness of performance in the hands of multiple operators and in different environments, which is especially impressive for a new technique with which the test sites had very little experience.

The PrepFiler™ kit has been developed specifically to improve the quantity and quality of DNA isolated from forensic samples to better support the entire forensic sample analysis process. We believe the results described in brief here demonstrate the ability of the PrepFiler™ kit to enable forensic laboratories to achieve better and more consistent results from a wide range of forensic samples than can be achieved with other currently available methods.

The PrepFiler™ kit for use with a manual protocol will be available for early access June 2008 with official release planned for late August/early September 2008. The PrepFiler™ kit for use with validated, automated protocols on the Tecan HID EVOLution™ System is anticipated to be released in September 2008.

For more information on maximizing the performance of DNA extraction from forensic samples using the PrepFiler™ kit, [click here](#) to view the article entitled "The Importance of DNA Extraction in the Forensic DNA Workflow" in this issue of *Forensic News*.