

## Forensic Technology Testing & Evaluation Report

**Final Report Date: 5 November 2009**

### Forensic Technology Testing & Evaluation Project

<b>Project Title:</b>	<b>Projected Start Date:</b>
Evaluation of Portable UV-VIS Spectrophotometer for Determination of Time Since Deposition (TSD) of Bloodstains at a Crime scene	4/27/2009
<b>Evaluation Type:</b> (Instrument, kit, procedure, product-to-product comparison study, etc.)	<b>Projected End Date:</b>
Instrument evaluation	10/31/2009
<b>Evaluation Team Leader:</b>	<b>Contact Telephone:</b>
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### Evaluation Team:

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### Manufacturer Information for product(s) being evaluated

Manufacturer	Address	Contact Person	Phone
Implen, Inc. Distributor: LabRepCo	101 Witmer Road Suite 700 Horsham, PA 19044	Joseph K Ray	267-495-6442

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## Evaluation Overview

### Evaluation Summary:

The ability to determine the time since deposition of a biological stain found at a crime scene could prove invaluable to investigators, defining the time frame in which the individual depositing the evidence was present. Although various methods to accomplish this in bloodstains have been proposed in the past, none has gained widespread use in the forensic community due primarily to limitations in the predictive power of the test and poor analytical sensitivities.

We have identified a novel hypsochromic shift (shift to shorter wavelengths) of the Soret band of hemoglobin that allows for bloodstains differing in age by minutes, hours, days, weeks and months to be distinguished. The resolution and sensitivity of this method make it particularly suited for use with forensic casework bloodstains. However, for this method to be of greater use in forensic casework, the ability to perform such measurements at the scene could be useful. This would allow investigators to identify if a stain was blood and how old the stain was. This could provide valuable information regarding what samples to collect at the scene for further analysis. In order to do this, a portable spectrophotometer would have to be available at the crime scene. Recently, a portable spectrophotometer has become commercially available from Implen called the NanoPhotometer™. The instrument has no moving parts making it more durable during transport to a crime scene and weighs less than 10 lbs. Initial data collected using this instrument while on loan indicated its potential utility at crime scenes. However, further validation of the instrument needs to be performed in order to determine its suitability for point-of-use deployment.

### Experimental Design:

(Outline of the procedure for evaluation)

Aim 1: Optimization of TSD measurements using the Implen NanoPhotometer™

Aim 2: Evaluation of the NanoPhotometer™ with forensic specimens (degraded samples, mock casework)

Aim 3: Technology transfer to NFSTC for use in the DOD/DTRA Deployable Forensic Laboratory

**Product(s) Specifications:**

**Brief description of Product(s)/Technology/Procedure being evaluated:**

Product Name(s)	Model Number:	Serial/Lot Number:	Dimensions:
Implen NanoPhotometer™ UV/VIS spectrophotometer	B-80-3004-31	1686	140 mm X 380 mm
	<b>Cost:</b>	<b>Weight:</b>	<b>Power Req.:</b>
	<b>\$10,000.00</b>	<b>&lt; 4.5 kg</b>	<b>90-250V, (50 Hz/60 Hz), Max 30VA</b>
<b>Storage Conditions</b>	<b>Room Temperature</b>		
<b>Operational Conditions:</b>	<b>Room Temperature, Natural Air, No fans</b>		
<b>Associated costs:</b> <i>(consumables, maintenance, etc.)</i>	<b>\$10,000</b>		

**Photo/Image of Product(s):**




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**Evaluation Instrumentation**

**Instrument Setup Performed by:**

- Manufacturer  
 Manufacturer and Evaluator(s)  
 Evaluator(s) Only

**Instrument Setup Comments:**

Installation of software was very straightforward. No challenges were encountered.

**Level of Operator Knowledge as Set by Manufacturer:**

Non-Scientist

Technician

Scientist

**Standards, Controls and Samples Used in Evaluation:**

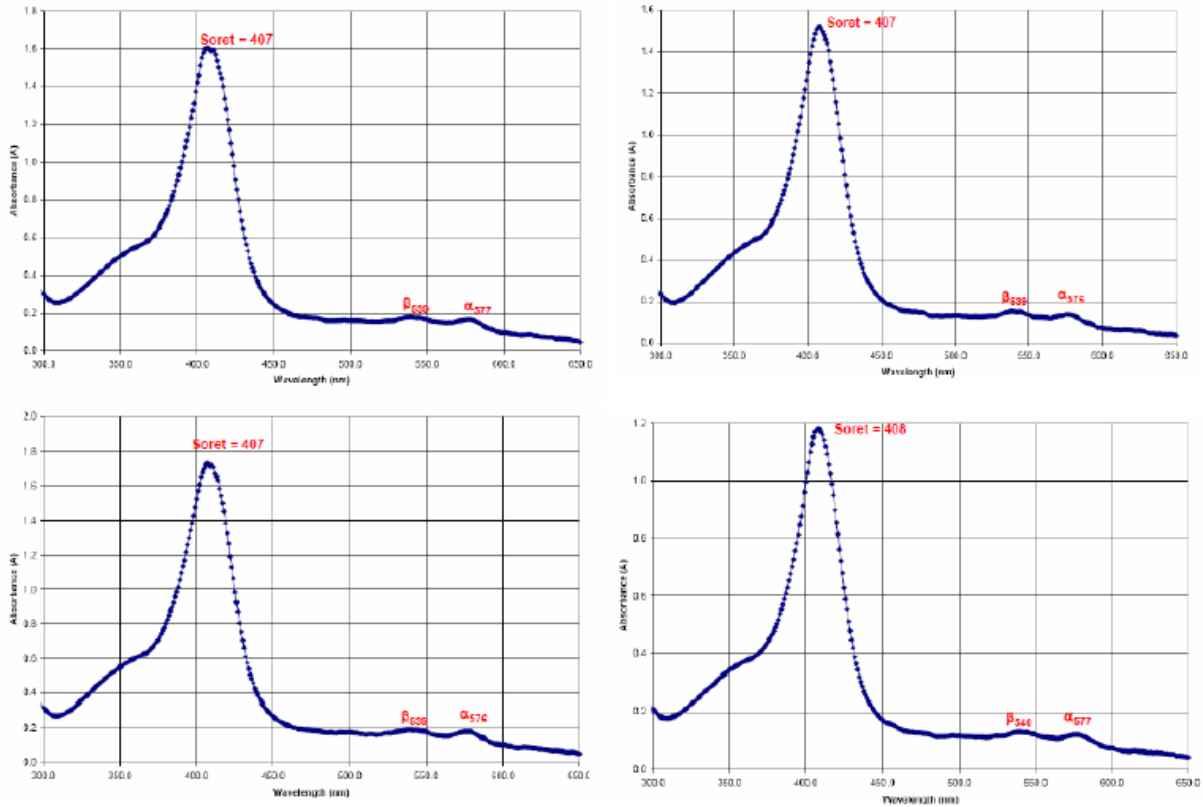
The instrument was delivered from LabRepCo to the NCFS laboratory. The following components were provided with the NanoPhotometer:

- NanoPhotometer
- USB cable and power supply
- Cuvette with 0.2 and 1 mm lids
- Thermal printer paper
- Protective cover
- Instruction manual
- Software CD (purchased and sent separately)

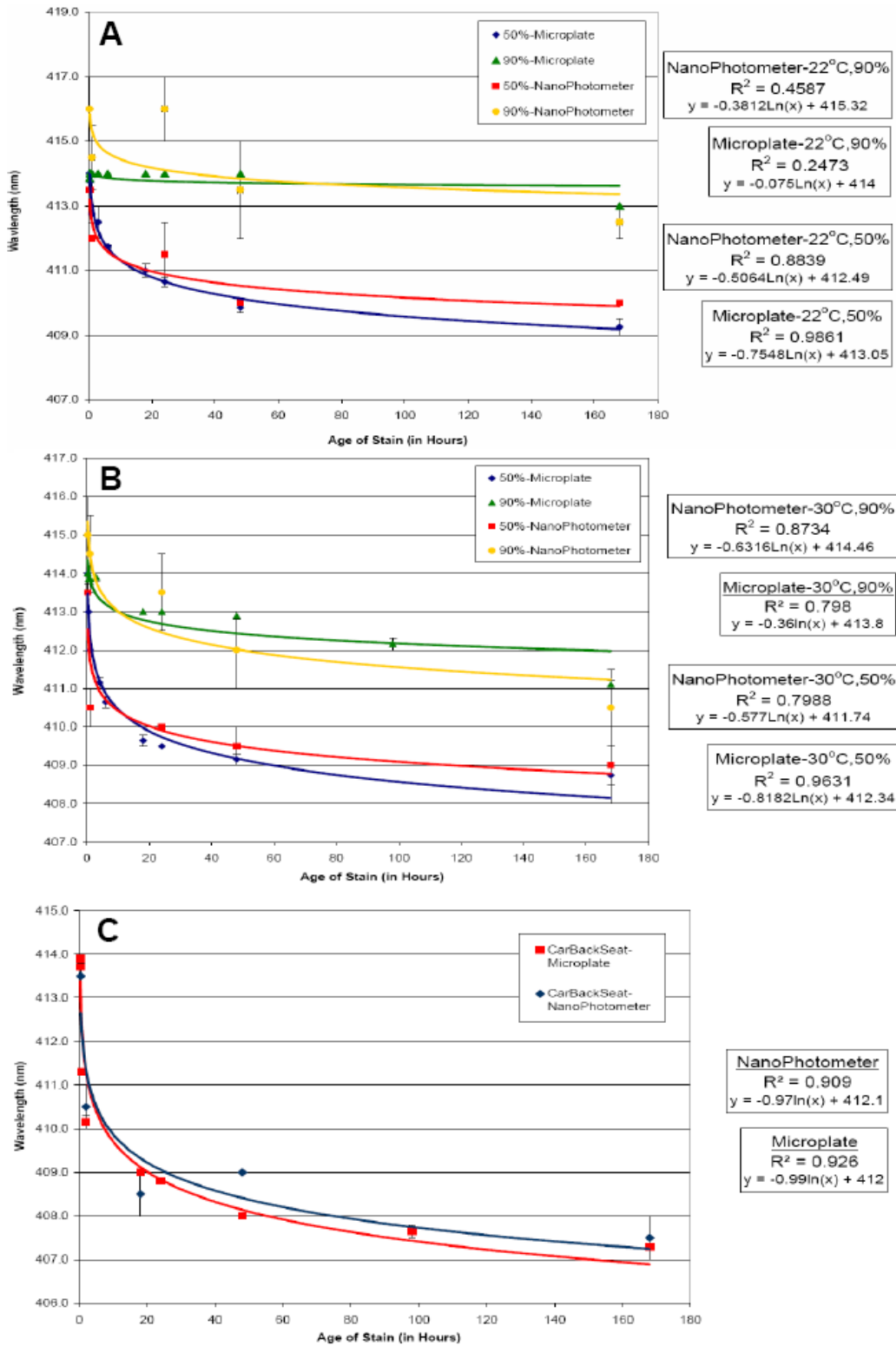
The instrument was placed on a laboratory benchtop and the instrument was plugged into a standard outlet and connected to a desktop computer placed next to the instrument. The software was installed to the attached computer. After powering on the instrument, it takes approximately 10 seconds for it to run required calibration checks and is then ready for use. The software takes approximately 15-30 seconds to recognize the instrument once the software is launched.

## Post-Evaluation Findings Results of Evaluation (Tables, Graphs)

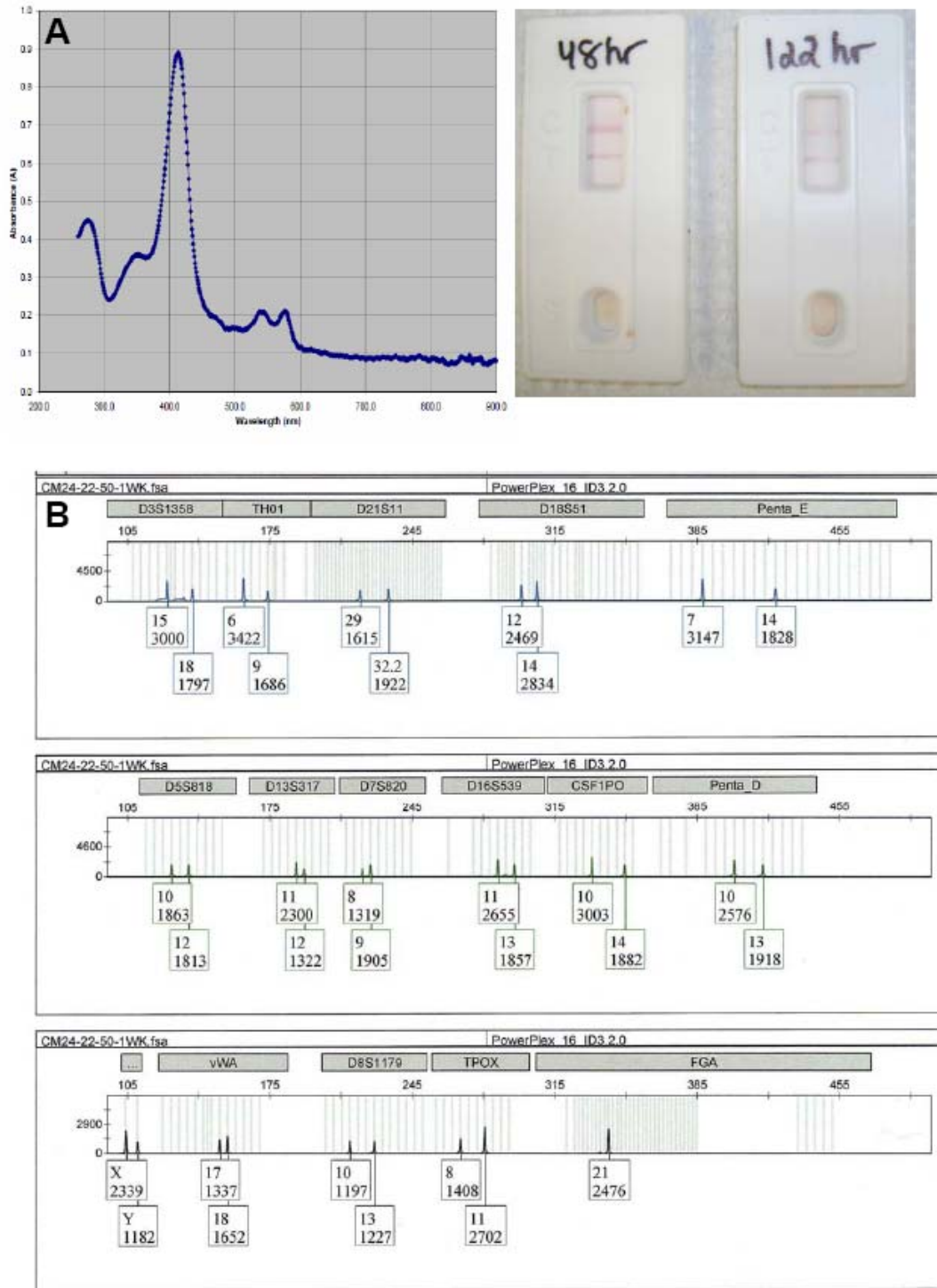
Figure 1. Spectral Profiles Obtained From 1/4 Bloodstains Using the NanoPhotometer



**Figure 2. Comparison of TSD Measurements using the BioTek® Synergy 2 Microplate Reader and the Implen NanoPhotometer**



**Figure 3. Confirmation of the Presence of Blood and Determination of the STR Profile of the Bloodstain Donor from an Individual TSD extract.**



**Table 1. DNA Recovery and STR Typing from TSD Bloodstain Extracts**

Donor	Exposure Condition	Length of Exposure	Quantity (ng/ $\mu$ l)	Amount Added to Amp	PowerPlex® 16 HS
			Quantifiler Human		
Female	Car back seat	1 week	0.23	1.2 ng	Full profile
Female	22°C, 50% humidity	1 week	0.20	2.0 ng	Full profile
Male	22°C, 50% humidity	1 week	0.06	624 pg	Full profile
Female	22°C, 80% humidity	1 week	0.04	446 pg	Full profile
Male	22°C, 80% humidity	1 week	0.02	228 pg	Full profile
Female	22°C, 90% humidity	1 week	0.02	120 pg	Full profile
Male	22°C, 90% humidity	1 week	0.05	485 pg	Full profile
Female	30°C, 50% humidity	1 week	0.02	152 pg	Full profile
Male	30°C, 50% humidity	1 week	0.02	186 pg	Full profile
Female	30°C, 90% humidity	1 week	0.58	2.0 ng	Full profile
Male	30°C, 90% humidity	1 week	0.05	456 pg	Full profile



### **Strengths/Results:**

#### **Strengths:**

- The NanoPhotometer is a portable, light weight UV spectrometer that could be used on-site at crime scenes by forensic laboratory/crime scene personnel (as integral hardware in a deployable “lab in a van” )
- The instrument is very easy to use and requires little training.
- The instrument requires very little cleaning and maintenance.
- The collected data can be printed directly from the instrument or connected to a computer to save the data directly into an Excel<sup>®</sup> document for further analysis.
- The data is generated within 20-30 seconds after the read option is selected.
- The instrument is capable of collecting full wavelength spectral scans from bloodstains with characteristic blood spectral profiles obtained (well defined  $\alpha$ ,  $\beta$  and Soret bands).
- The hypsochromic shift of the Soret band was observed in the data generated on the NanoPhotometer demonstrating its ability for use in time since deposition determinations in forensic analysis.
- The sensitivity of the instrument permits the use of small volumes of extract (0.7-3 $\mu$ l) to be used. The extract can be collected after the spectral scan is performed if needed for further analysis.
- The NanoPhotometer does not require any expensive consumables beyond the initial purchase.
- The instrument could be used for other purposes in the laboratory as it has additional functional applications: concentration determinations, multi-wavelength scans, absorbance measurements, kinetic assays, standard curves.

#### **Results:**

- We demonstrate the use of a portable spectrophotometer (NanoPhotometer) capable of performing TSD measurements “on-site” for possible future use in forensic casework.
- Optimization experiments were performed in order to determine the sensitivity of the instrument and the most suitable extraction conditions. Bloodstains of various sizes (whole,  $\frac{1}{2}$ , and  $\frac{1}{4}$  50  $\mu$ l) were extracted in various volumes of 0.2M Tris-HCl (50-500 $\mu$ l) for varying lengths of time (~1 minute, 15 minutes, 1 hour, 3 hours, 6 hours, and overnight). From each of these extracts, a range of input volumes onto the NanoPhotometer were tested (0.7-4 $\mu$ l). From these initial experiments it was determined that as little as a  $\frac{1}{4}$  of a 50 $\mu$ l bloodstain (~12.5 $\mu$ l) was sufficient for analysis. An immediate extraction with only gentle agitation of the bloodstain was also sufficient. It was determined that 3 $\mu$ l of the extract should be added to the NanoPhotometer for analysis. Refer to Figure 1 for results.
- The Soret band hypsochromic shift assay could be performed on the NanoPhotometer using the optimized conditions in order to determine the time since deposition of dried forensic bloodstains. Bloodstains stored at 22°C (50% and 90% humidity), 30°C (50% and 90% humidity) and bloodstains placed on the floor in the back seat of a car were analyzed using the NanoPhotometer and the hypsochromic shift results were compared to those obtained using the microplate reader. Similar  $\lambda_{\text{maxSoret}}$  values and linear regression functions were obtained for the samples using both instruments. Only slight variations were observed for the car back seat samples which more accurately represent the types of samples that may be encountered in forensic casework investigations. Refer to Figure 2 for results.
- In addition to a determination of the time since deposition of the bloodstains, the

presence of blood is simultaneously confirmed using this assay as a result of the characteristic blood spectral profile that is obtained (Figure 3A, left panel). However, additional tests were performed in order to confirm the presence of blood using methods routinely used in operational crime laboratories. We performed all additional testing using aliquots of the same extract used for the initial analysis on the NanoPhotometer so as not to consume additional sample in the preparation of multiple extracts. The ABACard HemaTrace test was used to confirm the presence of blood. Positive results for all samples tested were obtained. Refer to Figure 3A, right panel for an example of the positive ABACard Hema Trace results.

- We also wanted to determine if additional molecular-based testing could be performed on the remaining extract. We performed a DNA/RNA co-extraction using the Qiagen AllPrep Mini kit in order to isolate DNA for autosomal STR analysis and to isolate RNA for mRNA profiling. Varying amounts of DNA were obtained from the bloodstains tests (Refer to Table 1), but all samples resulted in the recovery of full autosomal STR profiles (Refer to Figure 3B). While a positive blood result was obtained using mRNA profiling for a few samples (data not shown), the routine use of the mRNA profiling would require modifications to the existing buffer in order to provide greater stability for the RNA and prevent RNase activity.
- The results from the current work indicate the potential to obtain an assessment of the time since deposition of dried bloodstains on-site and to simultaneously obtain confirmation of the presence of blood and recover autosomal STR profiles of the donor of the stain from the same bloodstain extract.

#### **Areas for Improvement:**

- There were no significant areas for improvement with this technology.

#### **Limitations of Technology:**

- The resolution of the NanoPhotometer is in 1nm intervals. Our previous work performed on the BioTek Synergy 2 Microplate reader allowed for the use of 0.1nm interval. It is possible that the smaller interval range would allow for greater accuracy and allow for smaller differences in stain age to be observed. However, a portable unit with 0.1nm interval measurements is not available. The use of the NanoPhotometer with 1nm interval measurements allowed for differentiation of bloodstains differing in age by minutes, hours and days.

#### **Training Requirements:**

- A very minimal amount of training is required to operate; this instrument can be operated by both technical and non-technical personnel. The training could be performed in as little as 30 minutes.

#### **Health and Safety Issues:**

- There are no significant health or safety issues for the operation of this instrument.

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