

# Detection of Urine-Based Deer Lures to Mitigate CWD Transmission in Pennsylvania

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## ABSTRACT

The Pennsylvania Game Commission contacted Millersville University to perform tests to help combat the transmission and spread of Chronic Wasting Disease (CWD) via better detection of urine-based deer lures. The objective of this study was to use multiple methods and kits designed for detection of urine and blood to determine the best method of detection for urine-based deer lures. Methods included use of kits such as Uritrace<sup>®</sup>, Nite-Site<sup>™</sup> luminol, Hemascein<sup>®</sup> and Ultra-violet (UV) light. We found that no one technique yielded a positive for all urine-based deer lures. The UV light and Uritrace methods were the most effective and the UV light was the best technique in field-testing. The spread of CWD throughout the Commonwealth has been confirmed and these findings provide a foundation to further develop methods to detect urine-based deer lures to help mitigate the spread of CWD.

**Keywords:** Chronic Wasting Disease; detection; urine-based lure; white-tailed deer

Chronic Wasting Disease (CWD) is designated as a dangerous transmissible disease believed to be caused by prions, which are transmitted via bodily fluids of white-tailed deer (*Odocoileus virginianus*) (Cullingham et al. 2011). There are no known treatments for CWD infections and the spread of this disease has a potentially severe detrimental impact on wild and captive white-tailed deer (Almberg et al. 2011). White-tailed deer can become reservoirs for other diseases such as bovine tuberculosis (*Mycobacterium bovis*) and brucellosis (*Brucella abortus*) that can spread to local livestock (Conner et al. 2008, McShea 2012), also deer serve as an intermediate vector for diseases such as Rocky Mountain spotted fever (*Rickettsia rickettsii*) and Lyme disease (*Borrelia burgdorferi*) which can infect humans (Centers for Disease Control and Prevention 2011).

The first positive test for chronic wasting disease in Pennsylvania was from a captive held whitetail deer in the summer of 2012 (Commonwealth of Pennsylvania 2013). Since the identification of a pen-raised white-tailed

deer infected with CWD in Pennsylvania, a Disease Management Area (DMA) was established around the location of the infected deer, in both Adams and York Counties. The Pennsylvania Game Commission established the DMA due to concerns that CWD may spread to wild white-tailed deer herd populations in Pennsylvania (Commonwealth of Pennsylvania 2013).

An executive order given by the Pennsylvania Game Commission established several restrictions within the DMA, one of which was the prohibition of the possession and use of deer urine (Pennsylvania Game Commission 2013). Urine-based deer lures are used by white-tailed deer hunters to attract deer into designated areas for harvest. It is estimated that between 10 and 40% of Pennsylvania hunters have used urine-based deer lures to hunt deer (Pennsylvania Game Commission 2013). At the same time, commercial urine-based deer lures to attract white-tailed deer have become readily available to consumers at common retail sporting goods stores such as Wal-Mart, Bass Pro Shops, and Cabela's. However, urine-

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based deer lures have been known to be collected from domestic white-tailed deer herds, with the potential of an animal infected with CWD residing within the herd. This can lead to the risk of CWD infecting wild populations of white-tailed deer via hunter use of deer lures. Thus, the use of urine-based deer lures is prohibited within the DMA (Pennsylvania Game Commission 2013).

Due to this risk, the Pennsylvania Game Commission contacted Millersville University to investigate if urine-based deer lures can be identified in the field using forensic tests. Based on a review of scientific literature and a lack of published reports, we know of no other study that has conducted these types of tests. The objective of this study was to determine if there were reliable field based tests to detect urine-based deer lures. The Pennsylvania Game Commission could then use these tests as part of a CWD prevention strategy in Pennsylvania. Our null hypothesis was that none of our forensics tests would be able to detect the presence of urine-based deer lures.

## METHODS

All analyses took place in the Applied Conservation Lab on the Millersville University campus. Our field site was located on the Millersville University Biological Preserve located off Creek Drive on the Millersville Campus. We used the following forensics test kits to test for the presence of urine-based deer lures: the Nite-Site™ luminol kit which detects blood residues; Hemascein® which detects latent bloodstains; Uritrace® which detects human urine; and Urine Stain Ultraviolet Light (UV) which is normally used to detect cat and dog urine (Appendix A).

Each of the four forensics test kits (Nite-Site™ luminol, Uritrace®, Hemascein®, and Urine Stain UV) were used to test for the following urine-based deer lures: Code Blue® Doe Urine, Code Blue® Buck Urine, Code Red® Buck Urine, Code Blue® Scrape Mate, Hunter's Specialties Primetime® Doe Urine, Wildlife Research Center® Active-Scrape®, Wildlife Research Center® Doe In Estrus® and Wildlife Research Center® Trail's End® #307®. All urine-based deer lures were in liquid form and water based, except for the Wildlife Research Center® Trail's End® #307®, which was oil based. When testing in the lab, water

or oil was used as the negative control. Positive controls were also used and included human urine for the Uritrace® test and the UV tests, human blood for the Hemascein® test, and bovine blood for the Nite-Site™ luminol test. We followed all procedures as outlined by each test kits manual of instructions.

Uritrace® was specifically designed to test for presence of creatinine, a byproduct of muscle metabolism, found in urine. This test, common in forensics and crime scene settings (Akutsu et al. 2012), used a small device containing 2 wells into which 3 to 4 drops of the urine sample were placed. One well held the urine sample and the other held the negative control. The well with the urine sample was compared to the color of the well containing the negative control and a positive result was indicated by change in color intensity in the urine sample. Samples were assigned a number on a 0 to 3 scale, 0 representing absence of any glow and 3 representing the most intense color glow. This test was conducted directly for each urine-based lure and compared to the results seen with water (negative control) and human urine (positive control).

We used the Nite-Site™ luminol test as a forensic test to detect blood. Nite-Site™ combined 5-amino-2, 3-dihydro-1, 4-pthalazinedione free acid, sodium carbonate, and sodium perborate tetra hydrate which reacted with the heme group in the blood to produce a blue glow (Barni et al. 2007). This kit was used to detect presence of blood in each of the urine-based lure samples. About 2 ml of each of the eight urine samples were poured onto separate 111 mm watch glasses, including bovine blood, which was used as a positive control and water as a negative control. Once the Nite-Site™ kit was prepared, the mixture was sprayed onto the watch glasses under UV light and monitored immediately for the presence of a blue glow. Samples were assigned a number on a 0 to 3 scale, 0 representing absence of glow and 3 representing the most intense blue glow.

The Hemascein® test was designed for revealing latent bloodstains in crime scene settings. In this kit, uranine was mixed with water and underwent several redox reactions after addition of sodium peroxide, which caused the formation of fluorescein, which

normally fluoresces under a light source between 415 and 480 nm (Lowis et al. 2012). When prepared, the first solution containing the fluorescein was misted over each lure sample, approximately 2 ml in 111 mm watch glasses, as well as a human blood sample. A 3% hydrogen peroxide solution was then made and misted over the lures immediately after, followed by observations in low light using the provided blue light (within 415-480 nm) and the provided "No IR" orange filter goggles.

The UV test utilized an LED UV flashlight that was shone on eight approximate 2 ml samples in 111 mm watch glasses. Samples were assigned a number on a 0 to 3 scale, 0 representing no glow and 3 representing the most intense color glow. In a second test, approximately 2 ml of each of the urine types were poured on a sheet of untreated cotton and immediately tested for UV glow. The dried stains were tested again after about 24 hours. In a third UV test, urine types were blotted onto both leather and rubber boots and tested with a UV light. Another UV test was conducted on 10 cm squares of denim, onto which approximately 1ml of each urine type was dropped and tested, both immediately and after about 24 hours. A fifth UV test used Hunter's Specialties® Spike Wicks, which were dipped in each urine sample, labeled, immediately UV tested, and hung in the Millersville Biological Preserve for about 24 hours, after which they were tested again. The final UV test was also conducted outside in the Millersville Biological Preserve at dusk and the eight urine samples were poured on the ground in a simulated white-tailed deer scrape. The light was immediately shone on the samples. After about 24 hours, the samples in the scrape were tested again.

Both the Uritrace® test and UV light test were also conducted on 100% cotton t-shirts. Each urine-based lure was sprayed approximately 4 times on 10 cm squares of fabric and left to dry for 24 hours and analyzed using UV light. In the Uritrace® test, a 0.25 cm<sup>2</sup> square was cut from the center of each fabric stained and soaked with each urine sample in 100 µl of distilled water for 1.5

hours. The water was then extracted using a 200 µl pipette and dropped directly into the sample well on the Uritrace® device for each urine sample.

## RESULTS

### Uritrace

The Uritrace® test did detect human urine as designed, and also detected a few of the urine lure samples (Table 1). The human urine gave a deep red color in the test and was rated a 3. The closest to this positive control was the Code Blue® Buck urine, which yielded a less intense red-orange and Code Blue® Scrape Mate gave off an orange color. Both of these urines were rated a 2 (Table 1). Code Red® Buck urine and Primetime® Natural Doe gave a yellow-orange color and were rated 1 (Table 1). The rest (Code Blue® Doe, Active-Scrape®, Doe In Estrus®, Trail's End® #307®, and the negative control, water) showed no change in color, yielding a negative result of 0 (Table 1). The variation in colors could be correlated with how diluted each urine type is and further testing would be required for each urine type. The 100% cotton t-shirt extraction for urine yielded all negatives, a rating of 0, after extracting samples for Uritrace® testing (Table 1). This may have been due to the extraction procedure further diluting the urine samples.

### Nite-Site™

The Nite-Site™ test demonstrated that there was little or no blood in the urine samples, a rating of 0. The only glow was seen on the plate with bovine blood, which gave an intense blue glow and thus a rating of 3 (Table 1).

### UV Tests

In the UV test on the direct urine-based samples, each watch glass contained an approximate 2 ml sample of urine. Doe In Estrus® was the only sample yielding a 3, although all other samples glowed, yielding a 1 or 2. Water did not glow and was assigned 0. These differences in intensity could again be correlated with differences in urine dilution of each sample type (Table 1).

Table 1. Compiled results of all the forensic tests completed on urine-based deer lures.

<b>Deer Urine Lure Type</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>	<b>Water</b>	<b>Human Urine</b>	<b>Bovine Blood</b>
<b>Tests</b>											
Uritrace	0	1	1	2	0	0	2	0	0	3	N/A
Uritrace Extraction	0	0	0	0	0	0	0	0	0	N/A	N/A
Nite-Site	0	0	0	0	0	0	0	0	0	0	3
UV Watch Glass	1	1	1	2	2	3	2	2	0	1	N/A
UV Scrape <sup>a</sup>	0	0	0	1	1	1	1	2	0	N/A	N/A
UV on Cotton <sup>b</sup>	0	0	0	2	2	1	2	2	0	N/A	N/A
UV on Denim	0	0	0	0	0	0	0	0	0	N/A	N/A
UV on Leather	0	0	0	0	0	0	0	0	0	N/A	N/A
UV on Rubber	0	0	0	0	0	0	0	0	0	N/A	N/A
UV on Wicks	0	1	0	1	0	0	0	2	0	N/A	N/A

Note: <sup>a</sup> Cleared area on the forest floor; <sup>b</sup> Untreated cotton

A positive for the Uritrace<sup>®</sup> is any color darker than yellow (assigned a 1, 2, or 3 depending on intensity) and a negative is yellow (0). In the Urine Stain Ultraviolet Light (UV) tests, 0 means no glow and 3 is the most intense glow. In the Nite-Site<sup>™</sup> test, 0 means there is no glow and 1, 2, and 3 signify the presence of a blue glow, dependent on intensity, the result of blood presence. Letters at the top of the table correspond to the following urine-based deer lure types:

A: Code Blue<sup>®</sup> Scrape Mate; B: Code Blue<sup>®</sup> Buck; C: Hunter's Specialties Primetime<sup>®</sup> Doe; D: Code Red<sup>®</sup> Buck; E: Wildlife Research Center<sup>®</sup> Active Scrape<sup>®</sup>; F: Wildlife Research Center<sup>®</sup> Doe In Estrus<sup>®</sup>; G: Wildlife Research Center<sup>®</sup> Trail's End<sup>®</sup> #307<sup>®</sup>; H: Code Blue<sup>®</sup> Doe

The 100% cotton t-shirt UV test yielded all negatives, rating of 0. However, in the UV test for untreated cotton, the following intensities were recorded for each urine-based lure: Code Blue<sup>®</sup> Scrape Mate, Active-Scrape<sup>®</sup>, Code Blue<sup>®</sup> Buck, and Trail's End<sup>®</sup> #307<sup>®</sup> were given a 2 and Doe In Estrus<sup>®</sup> were given a 1. The rest (Code Blue<sup>®</sup> Doe, Code Red<sup>®</sup> Buck, and Primetime<sup>®</sup> Natural Doe) had no glow (Table 1). Those with a glow gave a light peach color. Similar results were reported 24, 48 and 72 hours after application, with color intensity remaining the same.

The denim test yielded no positive results, both after pouring the urine on immediately and after 24 hours, no glow was seen. The urine tested on the rubber boots left little residue visible to the naked eye, and under UV, no glow was detected. For the leather boot UV test, when the samples were still wet, some of the urine that had not soaked in the leather gave a glow. When all the urine dried, no glow could be seen on the leather for any

of the urine types (Table 1). Some of the urine had dripped down onto the foam rubber sole of the boots and a peach-colored glow could be seen. Thus, we believe many other types of fabrics (polyester, canvas, etc.) could be tested in the future.

Three of the urine samples (Code Blue<sup>®</sup> Scrape Mate, Code Blue<sup>®</sup> Buck, and Trail's End<sup>®</sup> #307<sup>®</sup>) yielded a peach glow under UV light immediately after the Hunter's Specialties<sup>®</sup> Spike Wicks were dipped, the Trail's End<sup>®</sup> #307<sup>®</sup> was assigned a 2 and Code Blue<sup>®</sup> Scrape Mate and Code Blue<sup>®</sup> Buck were assigned a 1 (Table 1, Figure 1). The same three gave off a peach glow at their respective intensities after 24 hours. Although not all the wicks yielded a glow under UV light, all urine samples left a stain ranging from pale yellow to medium brown under ambient light when both wet and dry, and the samples also had a strong odor. Detecting urine lures is obvious by the presence of a key wick when seen by the naked eye, and detected by scent (Figure

1). Thus, the ability to recognize different brands and shapes of wicks is also an effective method of detecting use of urine lures in field settings.



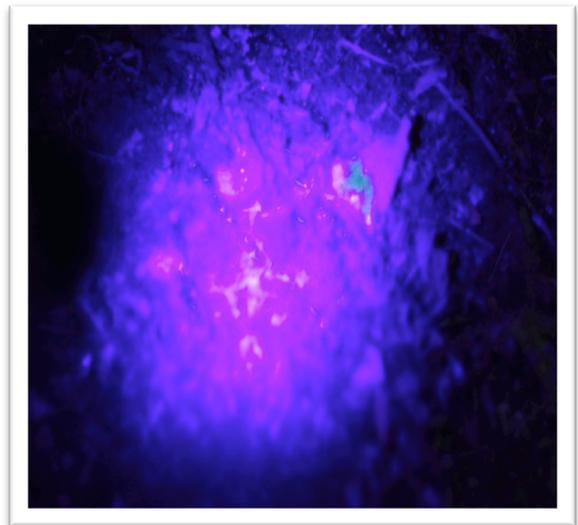
**Figure 1.** Hunter's Specialties® Spike Wicks with dried urine stains. Each number corresponds to a different urine type. 1: Code Blue® Scrape Mate; 2: Code Blue® Buck Urine; 3: Hunter's Specialties Primetime® Doe Urine; 4: Code Red® Buck; 5: Wildlife Research Center® Active-Scrape®; 6: Wildlife Research Center® Doe In Estrus®; 7: Wildlife Research Center® Trail's End® #307®; 8: Code Blue® Doe Urine. "W" denotes water. This demonstrates the ease of urine lure detection with the naked eye, as most lures stain the wick.

In the outdoor UV test, some glow could be seen immediately after application in Trail's End® #307®, Code Blue® Scrape Mate, and Active-Scrape®, Doe In Estrus®, and Code Blue® Buck urine samples. However, most of the urine-based lures immediately soaked into the ground and were only visible on leaves and sticks (Figure 2). After 24 hours, the site was revisited with the UV light, but only tiny flecks of dried urine could be seen on solid surfaces. The tiny flecks glowed peach and were assigned a 1. Trail's End® #307 glowed the most after 24 hours and was assigned a 2 (Table 1). These results are helpful in field situations and further UV testing of urine samples on leaf-covered ground should be conducted.

### Hemascein®

The Hemascein® test was the only test that yielded inconclusive results. The working

solution and the hydrogen peroxide solution were both sprayed carefully and lightly as repeatedly dictated in the instructions, but even the human blood sample did not fluoresce when exposed to blue light and viewed behind orange filter goggles. To attempt a positive control, the human blood was blotted onto a lab towel and lightly misted. After another negative result, the blot was allowed to dry and sprayed the next day, again yielding a negative result. The instructions say the test is designed to work on latent bloodstains. The blot was clearly visible and may have caused the negative result. Also, although misted carefully, both the working solution and the hydrogen peroxide solution dripped while spraying. The instructions say that too much of either solution can cause a negative result. Numerous trials of the Hemascein® test may be required to yield a positive test result, thus this may not be the best test to use for immediate field results.



**Figure 2.** Simulated scrape illuminated by UV light. Pictured (green-blue glow) is the Wildlife Research Center® Trail's End® #307® on a dead leaf, which yielded the best glow under UV light.

## DISCUSSION

We rejected our null hypothesis that none of the tests would yield a positive result for detecting urine-based deer lures. However, our tentative results suggest that each test has limitations in regards to detecting urine-based lures. The most promise in results was provided by the UV tests. However, we found

that urine-based lures using UV could not be detected in certain fabrics like leather and treated cotton shirts, but could be seen under UV light on solid ground surfaces (i.e., leaves, sticks) and on untreated cotton and shoe foam. A combination of methods (sight, smell, UV, identification of key wicks, and UriTrace® for those which yielded a positive) could be helpful to detect presence of most of these lures, whether on scrapes or on the different fabric material which tested positive. We are hopeful that the knowledge gained from these tests provides insight into better techniques to detect urine-based deer lures to help mitigate the spread of CWD throughout Pennsylvania.

Some commercial lures were collected from individual deer (Code Blue® Scrape Mate, Code Blue® Buck, Code Blue® Doe) and stated on their bottles that the urine will smell like a “real, single deer” and give a “true-to-life hunting experience”. On the back of the three Wildlife Research Center® products, a guarantee stated that the urine types were sourced from deer tested for (and free of) CWD. However, the only way to ensure that a deer is free of CWD is to test its brain tissue, which would require the deer to be deceased (Williams et al. 2002). In addition, the maximum disease course for CWD (time from exposure to end-stage clinical disease) is not known, but can exceed 25 months in experimentally infected deer (Williams et al. 2002). Therefore, we recommend that the Pennsylvania Game Commission should continue to ban urine-based deer lures on Pennsylvania DMAs.

#### ACKNOWLEDGEMENTS

We would like to thank Richard Palmer of the Pennsylvania Game Commission for his support of this project

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## **Appendix A**

### **Forensics Test Information**

Nite-Site<sup>TM</sup> luminol kit: [https://www.lynnpeavey.com/product\\_info.php?products\\_id=573](https://www.lynnpeavey.com/product_info.php?products_id=573)

Hemascein<sup>®</sup>: <http://www.abacusdiagnostics.com/hemascein.htm>

Uritrace<sup>®</sup>: <http://www.abacusdiagnostics.com/urine.htm>

Urine Stain Ultraviolet Light:

[https://www.lynnpeavey.com/product\\_info.php?cPath=22\\_37&products\\_id=1099](https://www.lynnpeavey.com/product_info.php?cPath=22_37&products_id=1099)