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DMAC Specificity for Human Urine Stains as Biological Evidence

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Abstract:

Urine detection in criminal casework is important for cases such as sexual abuse, physical assault and child abuse. To aid in the recognition of urine stains, a colorimetric test using p-dimethylaminocinnamaldehyde has been developed as a screening tool for positive presumptive body fluid stains that may have the component of urea present. It is important to know the level of specificity the DMAC reagent will give when testing unidentified stains on clothing and bedding or other forms of evidence. This study reports on the possibility of misidentifying a stain as possible urine when using an alternate light source in combination with DMAC. The rate of misidentification (false positives) was 2 of 9 body product samples (22.2%), 0 of 5 mascara samples (0%), 0 of 21 energy drinks (0%) and 0 of 16 food and drink substances (0%). This study shows that the only samples tested that yielded false positive results were moisturizers that contained urea. Semen and sometimes saliva samples also yielded false positive results.

Keywords: *DMAC, urine, forensic biology, presumptive test*

1. INTRODUCTION

Forensic biology relates to the study of biological materials left behind at a crime scene. Some biologicals such as body fluids will fluoresce due to various protein properties. The first step in evidence collection at the forensic biology laboratory is to screen items of clothing or bedding for body fluid identification. This research is specifically focused on the identification of urine as it is frequently identified in sexual assault and child abuse cases. Urine has a characteristic odor when fresh, but it may dissipate as the stain ages.

The color of the stain due to urine concentration also may vary. Fluorescence is often observed with an alternate light source (ALS) (ultraviolet and blue spectrum) at the perimeter of the stain due to the urea, creatinine and phosphorous components. Alternate light sources are commonly used for the generalized presumptive identification of possible biological stains based on fluorescence as semen and saliva fluoresce too.

Urine is mainly composed of water, urea, creatinine, uric acid, chloride, sodium, potassium, sulphate, ammonium, phosphate, and other ions in very small amounts. There are two main colorimetric presumptive tests that are used by forensic biologists to test for urine stains on biological evidence. One test that is commonly used is the Jaffe Test. This tests the sample for creatinine, a waste product of creatine that is removed from the body through urine. This test was created in 1886 by Max Jaffe after he discovered a reaction of creatinine with picric acid in an alkaline environment that turned bright yellow to orange in solution for a positive result [1].

Another colorimetric presumptive test that is commonly used is the DMAC test [2]. It reacts with urea, another waste product present in urine. The reagent used in the DMAC test is also known as p-dimethylaminocinnamaldehyde, which is an aromatic hydrocarbon that yields a pink color as a positive test reaction for the presence of urea.

There are many substances that can create a urine-like stain in appearance without being urine. Some examples of stains that look like urine could be energy drinks, apple juice and tea. In forensic biology, any stain with proper appearance would be further tested with an alternate light source for fluorescence and then with a presumptive colorimetric test for possible

body fluid identification [3-9]. This article will focus on the DMAC presumptive test and research the false positive substances that may yield both fluorescence and/or a positive DMAC reaction. Since false positives may occur, it is important to identify those substances to establish the potential for those to exist at the crime scene. DMAC is known to be a presumptive test because it will react with any substance that may contain the target substance of urea. There are multiple facial products that contain small amounts of urea that could potentially produce a false positive result for urine. Some of these products include eye creams, facial moisturizers, acne treatments, and aftershave [2].

This research examines the potential for false identification of stains with presumptive DMAC testing.

2. Materials And Methods

Clean unused socks were prepared by placing a sample with a gloved fingertip on the surface in an approximate 1 x 1 cm square and examined both wet and dry (Figure 1A). The alternate light source tests were performed with an UltraLite One with UV Magnum (405 nm) alternate light source. Stains that were positive fluoresced with a faint white to yellow fluorescence while being viewed through amber colored goggles using the ultra-violet light source (Thomas Scientific, Swedesboro, NJ) (Figure 1B).

The DMAC reagent (Sigma-Aldrich, St. Louis, MO) was prepared at 0.1% w/v by dissolving the appropriate amount of DMAC (1 mg) in 100 mL of molecular biology grade ethanol (Thermo Fisher Scientific, Waltham, MA). The DMAC test reagent was freshly prepared on the day of testing by adding 27 mL of the resulting solution to 3 mL of concentrated HCl (Thermo Fisher Scientific, Waltham, MA). Each stain was cut and placed on a spot plate. 1-2 drops of freshly prepared DMAC reagent were added and the color change if present was recorded within 5 minutes. Urine-stained cotton swatches were used as a positive control.

Negative controls were unstained cotton swatches as a substrate control. Experiments were reproduced on three different days and scored for color intensity using a 0 - 4 scale with 4 being the most intense color reaction.

The tested substances included:

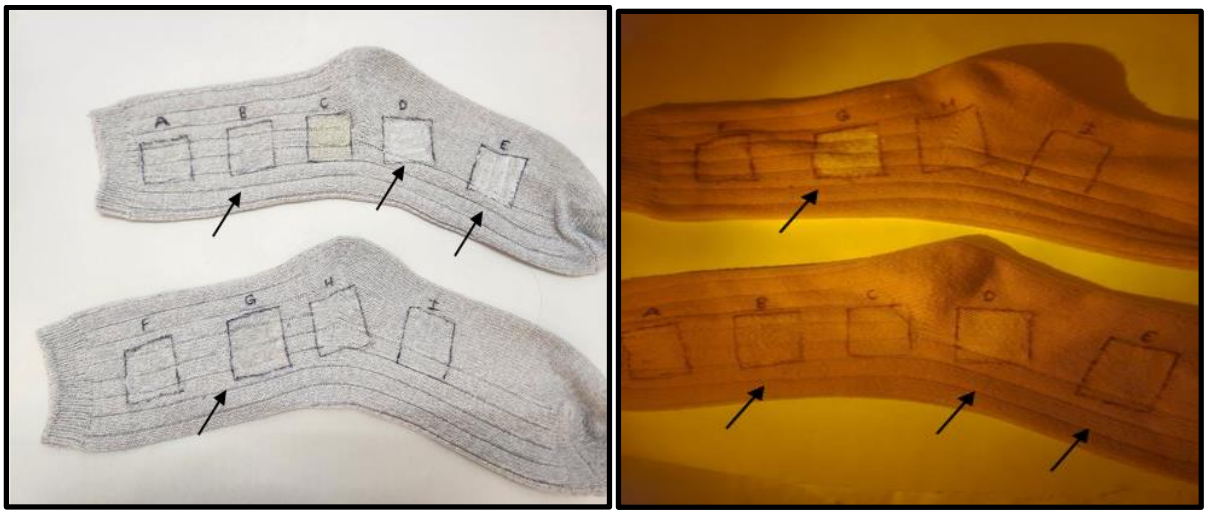
- Urine (positive control)
- CeraVe Psoriasis Moisturizing Cream
- Cetaphil Daily Smoothing Moisturizer for Rough and Bumpy Skin
- Clinique Dramatically Different Moisturizing Lotion
- The Ordinary Natural Moisturizing Factors + HA
- Pond's Dry Skin Cream Facial Moisturizer
- Aveeno Daily Moisturizing Face Cream for Dry Skin
- Cetaphil Gentile Clear Mattifying Acne Moisturizer
- CeraVe Daily Moisturizing Lotion for Normal to Dry Skin
- Neutrogena Daily Facial Moisturizer for Sensitive and Reactive Skin
- Saliva (body fluid)
- Semen (body fluid)

3. Results And Discussion

The clean socks show the color of the test substances which range from clear to yellow in color. When wet, stains D, E and G fluoresced under ultra-violet light (Table 1). When dry, stains B, D, E and G fluoresced (Table 1). Semen stains and sometimes saliva stains also exhibited fluorescence when dry as well as DMAC reactivity (data not shown). This is expected as semen and urine travel the same path in the male urethra and therefore, urea may also be mixed with semen (Figure 2A).

The faint reaction with saliva stains may be due to food components that cross react with DMAC reagent. Figure 2B shows the positive DMAC reagent color change to pink for four of the tested substances [CeraVe Psoriasis Moisturizing Cream (A), Cetaphil Daily Smoothing Moisturizer for Rough and Bumpy Skin (B), Clinique Dramatically Different Moisturizing Lotion (C), The Ordinary Natural Moisturizing Factors +HA (D)].

The top row of the plate has a positive control cotton swatch for urine (left), a substrate control cotton swatch with no urine but with DMAC (middle) and a substrate control cotton swatch with urine but no DMAC (right).



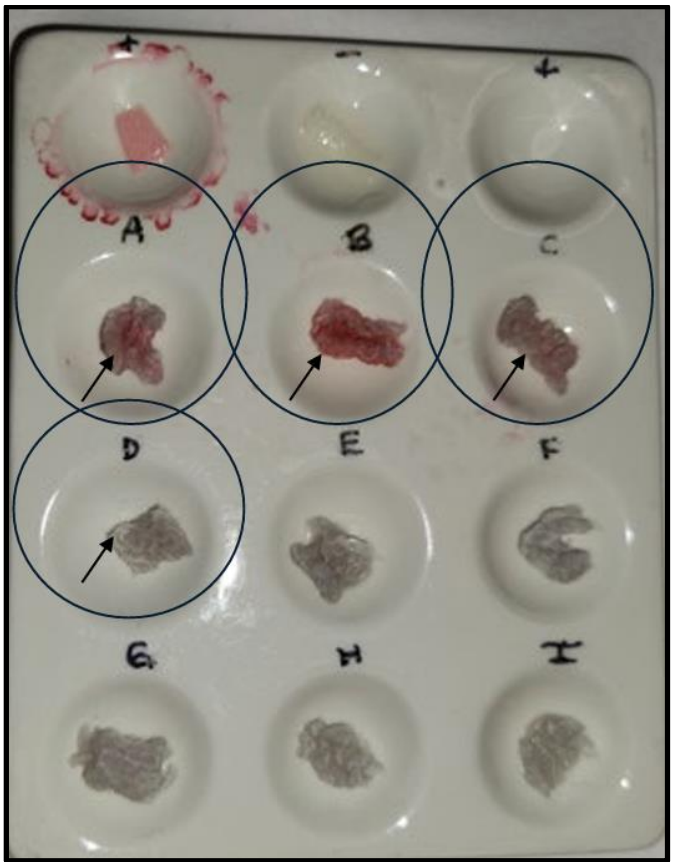
A B

Figure 1. *A. Prepared stains on socks and B. fluorescence of dried stains using ultra-violet light. Samples: CeraVe Psoriasis Moisturizing Cream (A), Cetaphil Daily Smoothing Moisturizer for Rough and Bumpy Skin (B), Clinique Dramatically Different Moisturizing Lotion (C), The Ordinary Natural Moisturizing Factors +HA (D), Pond's Dry Skin Cream Facial Moisturizer (E), Aveeno Daily Moisturizing Face Cream for Dry Skin (F), Cetaphil Gentile Clear Mattifying Acne Moisturizer (G), CeraVe Daily Moisturizing Lotion for Normal to Dry Skin (H), Neutrogena Daily Facial Moisturizer for Sensitive and Reactive Skin (I).*

Table 1. *Fluorescence results for stains illuminated with an UltraLite One with UV Magnum (405 nm) alternate light source (Thomas Scientific, Swedesboro, NJ).*

Sample	Florescence (wet)	Florescence (dry)
A	No	No
B	No	Yes(red glasses)
C	No	No
D	Yes	Yes
E	Yes	Yes
F	No	No
G	Yes	Yes
H	No	No
I	no	No

A



B

Figure 2. *A. Semen yields a false positive result, and this is likely due to delivery via the male urethra. The urethra carries both urine to the bladder for excretion and semen for ejaculation. B. DMAC reagent results for stains A – I. This colorimetric test turns pink for positive results. Stains A – C were strong positive for the presence of urea while stain D was a very faint positive reaction.*

The DMAC reaction was scored visually with a ranking score of 0 for no color change, 1 for a faint barely visible pink color change, 2 for a light pink color change, 3 for a medium pink color change and 4 for a strong hot pink color change. The range of color can be observed in Figure 2B where samples A and B are scored as 4, sample C is scored as 3, positive control is scored as 2, and sample D is scored as 1 for color change reactivity. Table 2 shows the results of scoring replicate spot plate tests over a three-day trial.

Table 2. *DMAC results for samples stains (A – I).*

Sample	DMAC Day One	DMAC Day Two	DMAC Day Three
A	4	4	4
B	4+	4+	4+
C	4	3	3
D	2	1	0
E	0	0	0
F	0	0	0
G	0	0	0
H	0	0	0
I	0	0	0
Saliva	2	0	0
Semen	1	0	0

Additional research on five different brands of mascara (Milani, Loreal, Essence, Maybelline, and Covergirl) showed no false positive results for ALS screening and DMAC testing (data not

shown). A third experiment examining energy drink stains on cotton socks resulted in one brand exhibiting fluorescence and two brands showed an unexpected blue color result. The samples tested were: Sparkling Ice: Berry Blast, Alani Nu: Kimade, Prime: Strawberry Watermelon, Bucked Up: Miami, Celsius: Mango Passionfruit, Monster: Ultra Fiesta Mango, Beyond Raw: Watermelon, C4: Skittles, Red Bull: Strawberry Apricot, Ghost: Orange Cream, Bang: Blue Razz, Gorilla Mind: Bombsicle, Reign: White Gummy Bear, GFuel: Hype Sauce, Monster: Original, Red Bull: Original, Rock Star: Fruit Punch, Tru: Orange Mango, Guayaki: Bluephoria, Bloom: Raspberry Lemon, and V8Plus: Pomegranate Blueberry. The Rock Star: Fruit Punch sample fluoresced with the alternate light source (UltraLite One with UV Magnum). Interestingly, Alani Nu: Kimade and Rock Star: Fruit Punch turned blue instead of pink with the addition of DMAC reagent. Therefore, we concluded that no energy drink stains would exhibit both fluorescence and a pink color change with DMAC based on this study and would not be mistaken for urine with these presumptive test methods.

Experiment 4 examined additional stains on clothing to determine if drinks, proteins or sugars in general would cause a false positive result with the ALS and DMAC reagent presumptive screening tests. The items tested were the following: SToK Un-Sweet Black Cold Bew Coffee, Pure Leaf Unsweetened Tea, Heinz Apple Cider Vinegar, Mountain Dew, Red Bull, Cabot Creamery Butter, Land O Lakes Margarine, Hellman's Mayo, Heinz Mustard, Prano Alfredo Sauce, Garelick Whole Milk, Sundae Shoppe Light Vanilla Ice Cream, Great Value Classic Lemonade, Schweppes Ginger Ale, Ratio Protein Vanilla Yogurt, and Dole All Natural Pineapple Juice. The following yielded a false positive fluorescence with an alternate light source: mustard, yogurt, margarine, milk, ice cream, pineapple juice and baby food. No false positive color changes were observed with the DMAC test results. This indicates that high concentrations of proteins cannot cause false positives for DMAC and that DMAC can effectively distinguish between urine stains and food stains while an alternate light source cannot fully distinguish between these two categories.

4. Conclusion

The results show that common food and drink that may produce yellow or tan stains on clothing do not yield false positives with the combined ALS + DMAC presumptive screening tests. The results seen in this study show that two common moisturizers that contain urea may also react with the ALS to produce fluorescence when wet or dry and can also yield false positives with the DMAC reagent for urine detection. The results seen in the study show that the DMAC reagent commonly used in forensic laboratory settings reacts with other body fluids (semen, saliva) that also contain urea in trace amounts and confirms the results of a prior study [2]. The presumptive tests for urine provide important information regarding the examination of materials in multiple types of cases including sexual assault, child abuse, and felony assault of a prison guard. It is important to know and understand the accuracy of the tests being used and what types of other substances commonly found on clothing and bedding could yield a false positive with these presumptive test methods. In our survey of possible substances, no mascara brands, no energy drinks, and no common food stains would result in a false positive assessment when using both the ALS and the DMAC reagent in combination. The samples Cetaphil Daily Smoothing Moisturizer for Rough and Bumpy Skin and The Ordinary Natural Moisturizing Factors +HA were the only two urea containing moisturizers that were both fluorescent and also positive with the DMAC test. Only body fluids and moisturizers containing urea would yield a false positive result with ALS + DMAC screening methods based on our study.

5. DISCLOSURE

The authors declare that they have no conflict of interest.

6. ETHICAL COMPLIANCE

The authors have performed this research in an ethical manner. The positive control body fluid samples were donated from authorized University of New Haven sources.

7. ACKNOWLEDGEMENT

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