H88 The Detection of Insect Stains From Four Species of Necrophagous Flies on Household Materials Using Immunoassays

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Learning Overview: After attending this presentation, attendees will understand how insect stains can be differentiated from human body fluid stains using a confirmatory immunoassay.

Impact on the Forensic Science Community: The presentation will impact the forensic science community by describing a new method for identification of insect stains found at crime scenes that can be reliably used to distinguish insect contaminants from body fluid evidence.

Any interaction between an insect with a corpse or associated exuded body fluids has the potential to create trace evidence in the form of insect stains. In many instances, the chief culprits producing the contaminates are several species of necrophagous Diptera that feed on a wide range of human tissues. By definition, only one type of insect artifact is officially recognized by bloodstain pattern analysts: insect stains. The Scientific Working Group for Bloodstain Pattern Analysis (SWGSTAIN) has defined insect stains as those bloodstains produced as a result of insect activity.[1] This definition leaves open the possibility of producing insect stains by two methods: insect modification of existing bloodstains or the creation of new stains. It is the latter that is most frequently cited by forensic entomologists, since both regurgitation and fecal elimination can yield insect stains containing human blood. The reality is that necrophagous flies can produce stains or artifacts as a result of feeding on several types of fluids (e.g., blood, saliva, semen, vaginal fluids, decomposition fluids), which yield artifacts that vary widely in terms of shape, color, and size.[2-4] Additionally, deposition of artifacts is not restricted to just foraging adults, as post-feeding larvae and newly emerged adults have the potential to contaminate crime scenes with unique artifacts.

Despite claims that fly artifacts can be detected based on morphological features, alternate lighting, and presumptive chemical tests, few species have been tested by the reported methods for discernment and none have proven to be consistently reliable in distinguishing insect stains from human body stains.[5,6] In an effort to overcome deficiencies in current methods used for identification of insect stains, an immunoassay has been developed that utilizes polyclonal antisera (termed anti-md3) based on a unique cathepsin D-like proteinase found in some cyclorrhaphous Diptera.[7] The confirmatory immunoassay (dot blot) recognizes insect stains that contain fly digestive enzyme, specifically fly regurgitate and defecatory or fecal stains.

In this study, artifacts produced by four species of necrophagous flies (*Protophormia terraenovae*, *Calliphora vicina*, *Cynomya cadaverina*, and *Sarcophaga bullata*) were examined using the confirmatory immunoassay to determine if insect stains could be distinguished from a range of human body fluids (e.g., blood, semen, urine, saliva, and feces). Adult flies were fed *ad libitum* human blood, semen, urine, feces, or saliva for 24h at 25ºC and permitted to deposit artifacts on a range of household materials: ceramic tile, carpet (plush), t-shirt (cotton), wood block, and unfinished drywall. A lift technique was developed that permitted transfer of fly artifacts from the test materials to filter paper (Whatman #4 110 mm ∅) for dot blot analyses.

Artifact transfers were confirmed visually and with an Alternate Light Source (ALS) using a 450nm emission filter and an orange contrast filter. All species readily deposited artifacts on all test household materials regardless of diet consumed. Despite differences in texture and porosity of the household materials, artifacts of all species transferred to saturated filter paper (Dulbecco's PBS) with apparent equal efficiency based on visual inspection. With all fly species, anti-md3 sera bound to artifacts produced after feeding on semen, blood, feces, urine, and saliva. Binding appeared proportional to the size of the artifact transferred during the lifts. By contrast, none of the human fluids tested positive in the immunoassays nor did lifts from household materials not exposed to flies. There was no evidence of false positives with any of the fly species tested, regardless of diet consumed. Similarly, there was also no indication of false negatives with any of the dot blot assays. However, flies did deposit artifacts not derived from the digestive tract on the test materials that, as expected, did not yield positive reactions with the immunoassay. Such artifacts generally cannot be visually distinguished from regurgitate and defecatory stains and thus can yield results perceived as false negatives.

These observations suggest that immunoassays using anti-md3 sera coupled with a simple lift technique can be used effectively as a confirmatory assay to distinguish fly regurgitate and fecal stains from human body fluids. The new method overcomes the limitations of current techniques and can be performed reliably by anyone properly trained without the need of a forensic expert for consultation.

Reference(s):

1. SWGSTAIN, Scientific Working Group on Bloodstain Pattern Analysis. Recommended Terminology. *I.A.B.P.A. Newsletter 2008*, June 14-17.
2. Durdle, A., van Oorschot, R.A.H., Mitchell, R.J. The Morphology of Fecal and Regurgitation Artifacts Deposited by the Blow Fly *Lucilia cuprina* Fed a Diet of Human Blood. *J. Forensic Sciences* 2013; 58(4): 897-903.
3. Rivers, D.B., McGregor, A. Morphological Features of Regurgitate and Defecatory Stains Deposited by Five Species of Necrophagous Flies Are Influenced by Adult Diets and Body Size. *J. Forensic Sciences*, 2018; 63(1): 154-161.
4. Zuhu, R.M., Supriyani, M., Omar, B. Fly Artifact Documentation of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae)—A Forensically Important Blowfly Species in Malaysia. *Trop. Biomed.* 2008, 25(1), 17-22.
5. Striman, B., Fujikawa, A., Barksdale, L., Carter, D.O. Alteration of expirated Bloodstain Patterns by *Calliphora vicina* and *Lucilia sericata* (Diptera: Calliphoridae) Through Ingestion and Deposition of Artifacts. *J. Forensic Sciences* 2011; 56: S123-S127.
6. Durdle, A. Mitchell, R.J., van Oorschot, R.A.H. The Use of Forensic Tests to Distinguish Blowfly Artifacts From Human Blood, Semen, and Saliva. *J. Forensic Sciences* 2015; 60(2): 468-470.
7. Rivers, D.B., Acca, G., Fink, M., Brogan, R., Chen, D., Schoeffield, A. Distinction of Fly Artifacts From Human Blood Using Immunodetection. *J. Forensic Sciences* 2018; In press.

Insect Artifacts, Immunodetection, Forensic Entomology