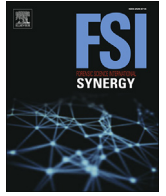




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Response to “Commentary on: Rivers DB et al. Immunoassay detection of fly artifacts produced by several species of necrophagous flies following feeding on human blood. Forensic Science International: Synergy 2019;1(1):1–10”



Artifacts produced by adult necrophagous flies can be highly variable in size, color, morphology, and frequency due to unique species behaviors, type of food consumed, size of meal, and length of time taken to consume the meal, as well as dependent on the physical surfaces on which they have been deposited [1–3]. At times, the contaminants produced by flies can potentially complicate crime scene investigations, such as those associated with bloodstain pattern analysis [4,5]. In fact, some investigators have called for studies focused on differentiating true bloodstains from insect artifacts, stating that such research “could greatly add to the knowledge of how to differentiate these stains” [5], and ... “a test to conclusively identify [insect] artifacts, in particular, would be highly beneficial” [1]. The research reported in Forensic Science International: Synergy 2019; 1(1): 1–10, is part of work supported by a National Institute of Justice grant aimed at responding to these recommendations by developing a confirmatory immunoassay that specifically distinguishes fly digestive artifacts from true human body fluid stains. The goal is for the assay to serve as a tool to complement other methods used by criminalists; not a replacement for other investigative tools currently in place.

In his Letter to the Editor, Ristenbatt [6] states that such a confirmatory test is unnecessary. He provides an informative outline depicting key characteristics of blood traces and “fly artifacts” that apparently are summarized from two sources [7,8], and then goes on to explain that based on these visual characteristics, along with contextual analysis, consideration of all relevant scene information, data, and physical evidence, and a criminalist with appropriate education, training, and experience, “fly artifacts” should not confound bloodstain pattern analysts [9]. We have no basis for disagreement in this regard, assuming that the information used for classification of fly artifacts is complete. Unfortunately, many of the salient features of fly artifacts he provided are either not complete or inaccurate in description. Part of the problem stems from the fact that descriptions of fly artifacts need to be placed in context to have meaning. Only two types of fly artifacts are referenced by Ristenbatt, regurgitate and defecatory. At least four types of insect stains are recognized in the literature [2,4,10] but as many as ten unique types of artifacts based on mechanism of production have been observed with necrophagous/saprophagous flies [11,12 and unpublished observations during our NIJ grant]. The shapes and color of fly artifacts provided by Ristenbatt are summarized from work of only three species of flies, all from the family

Calliphoridae, and in one of those studies [8], artifact morphology was based on *ad libitum* feeding on chicken not human blood. Work has now been completed on four additional species representing three families of Diptera [10], and the data has revealed that artifact color is even more varied than originally suspected. Yet most stains appear to be the color of the food source. As such, flies that feed predominantly on blood produce artifacts that, generally, are the color of blood. However, if decomposition fluids are consumed, then the insect stains typically are not the color of bloodstains. Additionally, this is dependent on species of fly, geographical differences within the same species, size of meal, duration of meal in fly gut, and substrate that the artifacts are deposited upon. For many species examined, “tails” are not associated with any type of artifact [10]. Even for some species reported to have defecatory tails, the production of such tails appears to be associated with specific geographical locations within the same species [10,13,14]. As to the description of regurgitate stains provided by Ristenbatt, cratered fly stains have only been reported for two species of calliphorids (*Chrysomya megacephala* and *Lucilia cuprina*), both under laboratory conditions [1,8], and has been attributed to be due to either the sucking action of mouthparts of flies that return to feed on the stain, or to drying on a smooth, non-porous surface. Overall, cratered stains are not commonly encountered [15], yet non-cratered regurgitate stains are frequently produced by at least five species of necrophagous flies [10].

In reality, too few fly species have been tested to come to any complete consensus on physical attributes of fly artifacts. Over 80,000 species of Diptera occur worldwide, with at least 16,000 species found within the United States [16]. Of these, roughly 7%, or just over 1000 species belong to the forensically important families Calliphoridae, Sarcophagidae, and Muscidae. Approximately 400–500 additional fly species belonging to the micro-dipteran families Fanniidae, Piophilidae, Phoridae, Psychodidae, Sepsidae, and Stratiomyidae are also of forensic significance dependent on the stage of body decomposition and location of the remains. The point is that hundreds of these necrophagous fly species feed on corpses and body fluids located indoors or outside, as well as colonize human remains found in any artificial situation imaginable [17]. Each of these fly species can leave behind evidence of their activity in the form of artifacts. In other words, less than 0.2% of all forensic species known in the United States have been examined by the reported methods for discerning fly artifacts from human bloodstains and other bodily fluids.

Is there a need for a confirmatory assay to distinguish fly

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artifacts from human body fluids? Certainly not for every scenario in which fly evidence is present at a crime scene. But at other times, the immunoassay developed has the potential to be useful over the course of an investigation. Ristenbatt [6] provides examples of “fly artifacts” in his Letter to the Editor in Figures 1 and 2. The stains shown are stated to be fly artifacts, apparently based on morphology and location. How did the author *confirm* that they were in fact derived from flies? This seems like a fair question since bloodstains are not confirmed based on visual analysis alone. Yet he implies that the stain identity is, indeed, known. In the absence of a confirmatory test or visual observation of fly deposition of regurgitate and/or feces, it seems unlikely that Ristenbatt would be able to state conclusively that the trace evidence in the figures that he cites are indeed fly artifacts.

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