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DEVELOPMENT OF A LATERAL FLOW TEST FOR BED BUG DETECTION

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Abstract Lateral flow strip tests are a cost-effective method for detecting specific proteins in biological samples, which can be performed in the field without specialized expertise. While most recognizable in the pregnancy tests, there are many other applications for lateral flow strip technology. The pest control industry has increasingly emphasized the importance of pest monitoring to reduce unnecessary applications, focus interventions into locations with active pest infestations, and to develop records of pest infestation. Due to their cryptic behavior, the detection of bed bugs often necessitates labor-intensive, time-consuming and invasive visual inspections. A lateral flow strip test for the detection of bed bugs would represent a novel use for a well-established technology, which can enable pest control operators to rapidly confirm the presence or absence of bed bugs in a room. In the current report, we present an effort to develop and calibrate the lateral flow test devices for the detection of a bed bug specific protein. A variety of bed bug residue samples were prepared by varying several parameters: bed bug infestation level (1 bed bug / 3 bed bugs), surface type (wood / fabric), feeding status (fed / unfed), and bed bug time-on-surface (1 d / 7 d). Using a prototype sensor and test strip, we examined how these variables influenced the detection of the bed bug specific proteins in the sample and to what degree. We will discuss how this lateral flow test device can be an effective tool to determine the presence or absence of bed bug proteins on a surface, providing highly credible evidence on bed bug infestations.

Key words *Cimex lectularius*, bed bug, detection, lateral flow strip.

INTRODUCTION

As haematophagous insects, bed bugs (*Cimex lectularius*, L.) feed exclusively on blood for survival, with nymphal instars requiring blood meals for development, and adult females requiring blood for reproduction (Harlan, 2006). When the infestation levels are relatively low, bed bugs are notoriously difficult to spot, due to their nocturnal biology and their tendency to hide in cracks and crevices out of sight from their host (Harlan, 2006). Because of this cryptic lifestyle, controlling bed bug infestations is also a difficult and labor-intensive process, often necessitating the use of chemical treatments or heat treatments. Currently, most of the services available for bed bug control are curative and remedial. These reactive approaches can be less effective in multi-family or high-occupancy housing settings in which bed bugs can easily transfer from infested units to un-infested units (Wang et al., 2009, 2010) through social contact (Wu et al., 2014) and shared spaces (Wang et al., 2010). If a pest control operator lacks access to infested rooms, the prolonged infestation can bloom out of control as the infested unit serves as a refuge for bed bugs (Wang and Cooper, 2011).

Proactive and early detection of bed bugs is crucial in maximizing control efforts, reducing the need for repeated visits and minimizing the likelihood of a chronic infestation (Wang and Cooper, 2011; Cooper et al., 2016ab). There are several bed bug detection devices currently available in the market today. These range from inexpensive 'passive monitors (no lure)' such as sticky traps and pitfall traps to more elaborate and expensive 'active monitors (with lure)'. Sticky traps are not effective in detecting bed bugs (Hottel et al., 2019) despite their widespread use (67.9% of PCOs use sticky traps for bed bug detection; Gangloff-Kaufmann et al., 2006). In contrast, pitfall traps are effective in detecting low-level bed bug infestations (Wang et al., 2009a, 2009b, 2010, 2011; Cooper et al., 2014, 2016a, 2016b). However, both sticky trap and pitfall trap require at least two separate visits by the pest control operator (one visit for installation and a separate visit to inspect traps). Passive monitors also require regular service (cleaning and/or relubrication), as dust and debris buildup can render both sticky traps and pitfall traps ineffective (Cooper et al., 2015). While active bed bug traps with lures generally entail greater cost, the detection rate of these

devices vary significantly (Wang et al., 2011; Sheele et al., 2016) and likely depends upon the design of the trap (Vernon and Gillespie, 1995) and the type of active lure being used (Singh et al., 2013).

Bed bug detection services are also commonly offered, such as human inspection by a pest control operator and the use of canine bed bug detection teams. Like the devices, these also vary in detection accuracy and cost. Detailed visual inspections (requiring 42-90 minutes per apartment) by an experienced pest control operator only yield a 52% accuracy rate (Wang et al., 2010; Pinto et al., 2007), and canine bed bug sniffing dogs vary significantly in their detection rate (44%) among teams and within teams over the span of several days (Cooper et al., 2014). No device or service has yet been able to achieve high levels of bed bug detection accuracy, combined with low cost, and real-time results.

Since its first commercial application in the widely available pregnancy tests, the lateral flow strip technology has found additional applications in the detection of pathological elements for the military (bio-defense), infection/contamination detection, presence of toxic compounds in food/feed, and presence of illicit drugs (Posthuma-Trumpie et al., 2008). These point-of-care devices are inexpensive to manufacture and simple to use; they enable accurate and robust biological testing in areas that lack access to scientific equipment.

In this paper, we describe the use of lateral flow strip technology for the detection of bed bugs. One of the major objectives of the research was to calibrate lateral flow strips for the accurate detection of bed bug residues. Several different surfaces containing bed bug residues were prepared by varying the number of bed bugs on the surface (1 vs 3 bed bugs), the feeding status of the bed bug(s) (fed vs unfed), the substrate type (fabric vs wood), and the amount of time the bed bugs spent on the substrate (1 day vs 1 week). Including no bed bug controls (clean fabric or wood surfaces without bed bug residue), a total of 18 different surfaces were sampled with special swabs. The swabs were then shipped to an external lateral flow assay laboratory where they were analyzed using a prototype sensor and test strip. The accuracy of the lateral flow strip device was determined in discriminating infested from non-infested (control) swabs.

METHODS AND MATERIALS

Treatments

We exposed one or three, fed (1 or 2 days post-feeding) or unfed (>7 days post-feeding) bed bugs on either fabric (cotton) or wooden surfaces (bass wood panel) for either 1 or 7 days. The bed bugs were confined within a circular area (23 mm in diameter) on the surface of substrates by covering them with 12-well cell culture plates (Corning Inc., Corning, NY). After removing bed bug from the substrates (with a brief flow of CO_2), the circular area was sampled with a swab. While gently pressing the side of swap against the substrate, a circular movement (2 rotations) was used to sample the entire area where the bed bug(s) might have contacted. Overall, we evaluated 18 different treatment combinations varying in bed bug density (2 levels), exposure time (2 levels), feeding status (2 levels), and substrate type (2 levels), including two controls (no bed bugs). Control swabs were prepared by swabbing either the fabric or wooden surface alone. Treatments and controls were replicated 10 times.

Swab samples coded with a unique identification number and processed at Lumos Diagnostics in Carlsbad, CA (https://lumosdiagnostics.com/). Lumos Diagnostics extracted each swab into individual buffer solution, which were then placed into the lateral flow assay device. The test was conducted in a blind fashion, as Lumos Diagnostics did not have the treatment assignments that each swab sample belonged to. After 5 minutes the lateral flow assay test was concluded, and sensor value was read. Higher sensor values indicate greater likelihood of the presence of bed bug residues collected on the swab. Our challenge was to identify the sensor value that most differentiates the control swabs (noise) from the infested swabs (signal); i.e., the signal threshold. Identifying this signal threshold would also enable the calculation of accuracy of the device.

Lateral Flow Strip Device

The lateral flow strip device uses a pair of optical sensors with an illumination source that is positioned between the test line and the control line on the cellulose lateral flow assay strip. These optical sensors calculate the reflectance of the light from the white cellulose strip. Decreased reflectance is correlated with increased sensor values from the optical sensors, which indicate that the protein/colloidal gold antibody complex have successfully attached to the region of the strip being analyzed; i.e., either the test line or control line.

Calculation Of Accuracy

Accuracy was calculated using the following formula:

Accuracy = (TP + TN) / (TP + TN + FP + FN)

TP = True positive (instances in which the test predicts positive and actual value is positive)

TN = True negative (instances in which the test predicts negative and actual value is negative)

FP = False positive (instances in which the test predicts positive and actual value is negative)

FN = False negative (instances in which the test predicts negative and actual value is positive)

Figure 1. was created by calculating the total accuracy of the swabs given a theoretical signal threshold, ranging from 0 to 400. For example, because signals from the electronic reader cannot be negative, a signal threshold of 0 would result in a 0 TN rate and a 0 FP rate. As the signal threshold increases in increments of 10, the accuracy is calculated until a theoretical maximum accuracy rate emerges.

Data Analysis

Data was analyzed using the R statistical program and Microsoft Excel. P-values less than or equal to 0.05 were regarded as significant. A one-way ANOVA was used to determine significance, and a Tukey's Post Hoc test was used to differentiate significance among treatments. F-tests were used to determine variance between treatments. For data shown in Figure 2, data failed to satisfy parametric assumptions of skewness, kurtosis, and heteroscedasticity, so a non-parametric Kruskal-Wallis test was performed using the Agricolae package in R.

RESULTS

The results indicate a theoretical maximum accuracy range of 93.1% and 93.3% at a signal threshold of 50 and 60, respectively (Figure 1). The swabs taken from control surfaces exhibited significantly lower sensor values compared to the swabs sampled from bed bug contaminated surfaces (Figure 2). At a signal threshold of 50, accuracy was 93.06%, true positive rate was 95.3%, true negative rate was 75%, false positive rate was 25%, and false negative rate was 4.6%. At a signal threshold of 60, accuracy was 93.33%, true positive rate was 93.75%, true negative rate was 90%, false positive rate was 10%, and false negative rate was 6.25%.

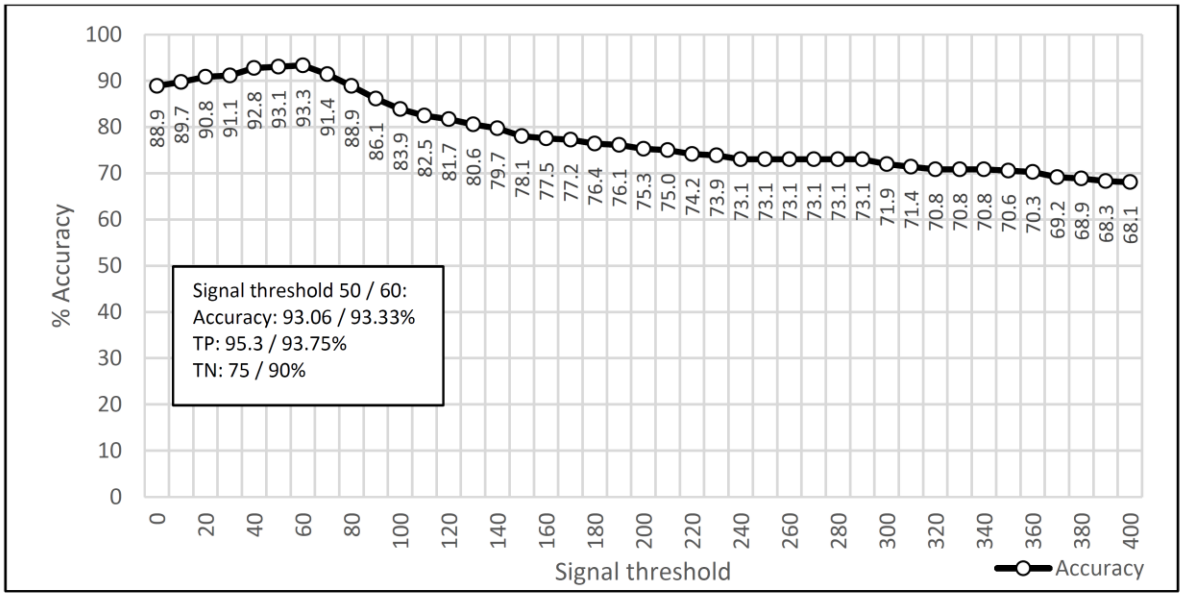

Figure 1. Calculation of accuracy given signal thresholds ranging from 0 to 400 (x-axis). More detailed breakdown of true positive / negative and false positive / negative is provided in the box for the two signal thresholds with the highest accuracy.

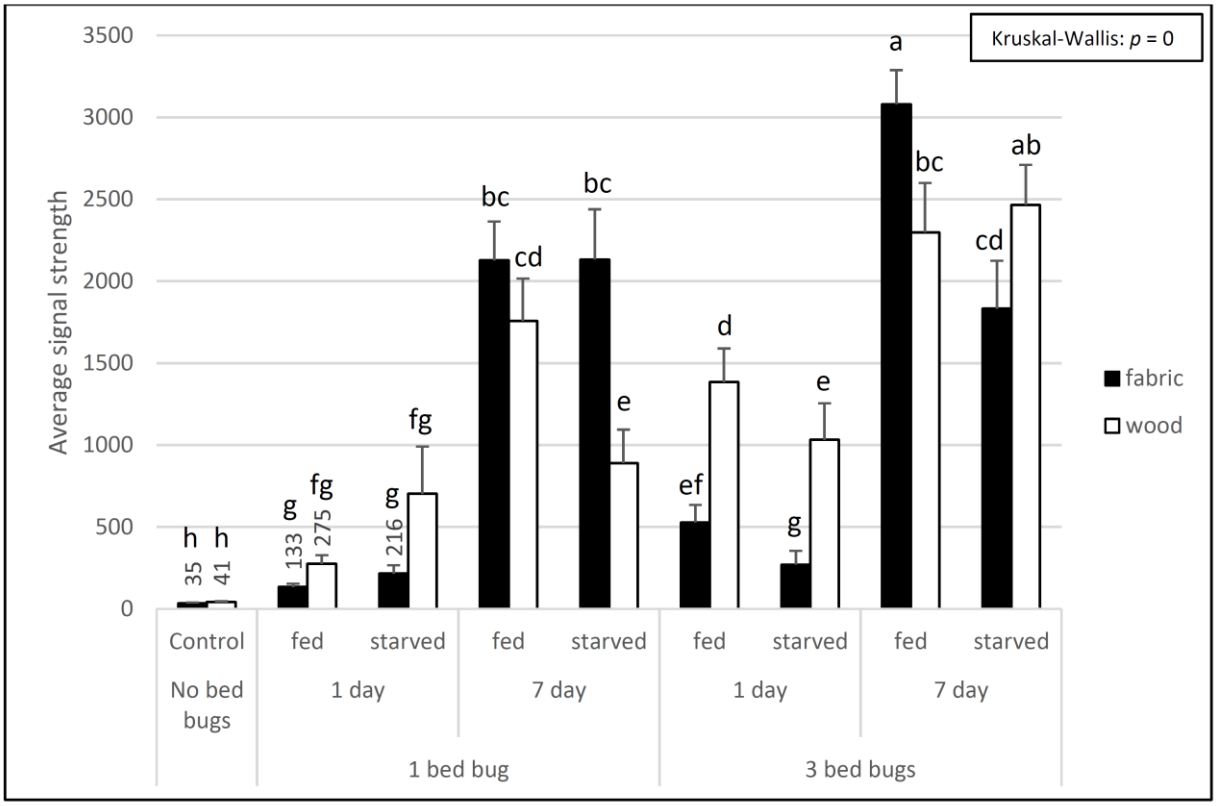

Figure 2. Signal differentiation among treatments (Standard error bars shown). Different letters indicate significant differences. Note that control samples are below the signal threshold of 60 and are significantly less than the other swab samples taken from bed bug infested surfaces.

Average Signal Strength Across All Treatments. Control swabs taken from fabric and wooden surfaces were significantly less (Kruskal-Wallis test: critical value = 243.6511, df = 17, $p = 0$) than swabs taken from infested surfaces (Figure 2).

Effect of Substrate on Sensor Values. Sensor values from swabs taken from fabric surfaces (mean = 1289.48; SE = 107.611; n = 160) were not significantly different (t-test equal variance: t-stat = -0.415, df = 318, $p = 0.677$) than sensor values taken from wooden surfaces (mean = 1350.3; SE = 99.1779; n = 160), although sensor values from wooden surfaces were slightly higher than sensor values taken from fabric surfaces.

Effect of time bed bugs spent on substrates. Sensor values of swabs sampled from 1-day infested surfaces (mean = 508.909; SE = 63.7637; n = 160) were significantly less (t-test unequal variance: t-stat = -12.58, df = 268, $p = 0$) than swabs taken from 7 day infested bed bug surfaces (mean = 2072.4; SE = 101.189; n = 160).

Effect of bed bug feeding status. Starved bed bugs produced surfaces that resulted in lower sensor values (mean = 1192.36, SE = 101.554, n = 160) than fed bed bugs (mean = 1447.415, SE = 104.45, n = 160), although this difference was not significant (t-test equal variance: t-stat = 1.75, df = 318, $p = 0.0809$).

Effect of bed bug density on sensor values. Sensor values of swabs sampled from control surfaces (mean = 37.89; SE = 3.07; n = 40) were significantly lower than swabs sampled from surfaces contaminated with 1 bed bug (mean = 1028.98; SE = 95.959; n = 160), which were also significantly lower than swabs sampled from surfaces contaminated with 3 bed bugs (mean = 1610.796; SE = 105.618; n = 160) (ANOVA, $p = 9.7e-12$, Tukey's HSD).

DISCUSSION

To achieve satisfactory bed bug control, pest control operators must be able to detect low-level bed bug infestations. If low-level infestations can be efficiently detected for early intervention, the potential for bed bug introduction into adjacent areas could be also minimized. Therefore, in order to develop a truly proactive bed bug management service, pest control operators must be able to detect even low-level infestations.

We find that lateral flow strip technology is extremely accurate (93.33% @ signal threshold of 60) in detecting even low-level bed bug residue on both fabric and wooden surfaces. It is important to note that a single starved bed bug can produce enough residue to be detected after just one day (Figure 2), demonstrating the sensitivity of the lateral flow strip technology. Control swabs of both fabric and wood substrates exhibited significantly lower signals than all the infested swabs (Figure 2). Our finding that sensor values taken from fabric surfaces did not vary significantly from sensor values taken from wooden surfaces is a positive result, as pest control operators can be expected to use a bed bug detection tool on a variety of surfaces; thus, sensor values and the likelihood of bed bug detection should not vary significantly among surfaces swabbed. Our findings that the number of bed bugs residing on a surface, and time spent on substrate, all affected the likelihood of bed bug detection, is a great boon to our confidence that lateral flow strip technology is sensitive enough to reliably detect even low-level bed bug infestations.

CONCLUSION

Detection of bed bugs will play a more important role in the future beyond the enhanced control of the pest. As the pace of technology increases and detection accuracy with technologically sophisticated tools also increase, we may find that our standards for pest-free environments are likewise elevated. It is interesting to postulate what might happen if general consumers such as hotel guests and travelers are given the ability to detect bed bugs with a high degree of accuracy; how might bed bug detection services and expectations be shaped by such a technology in the future?

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