

INSTRUCTIONS

"Developed in collaboration with entomologists at the University of Kentucky."



Brian Hirsch | 414.731.1663 | Brian.Hirsch@protectabed.com www.lightsoutbedbug.com | www.protectabed.com



Lab-In-A-Bag[™] Pesticide Efficacy Kit

The LightsOut Lab-in-A-Bag™ Pesticide Efficacy Kit was developed to help the pest management industry choose the most effective insecticides, especially when treating for bed bugs. It's the first tool of its kind to be marketed for this purpose. Much as a physician prescribes antibiotics, pest managers will now be able to select the most suitable products and methods to treat a customer's infestation.

Every Infestation is Different - No two bed bug infestations are identical in their susceptibility to insecticides. Studies conducted by the University of Kentucky and other institutions have found tremendous variation, with certain compounds performing well against some populations and poorly on others. Different formulations of the same compound can also vary in effectiveness, which can be further impacted by whether the substrate is fabric, wood, etc. Consequently, clients would benefit from knowing which products are most effective against their particular infestation.

Resistance is Rampant - Bed bugs are highly adept at becoming resistant to insecticides. Similar to the genetic 'arms race' between germs and medicine, resistance in bed bugs is widespread and becoming a global predicament. History has shown that these genetic changes in bed bugs can occur swiftly. In the case of DDT, for instance, some bed bug populations were already becoming resistant within the first few years of use. Bed bugs are teeming with genetic mechanisms to resist modern insecticides as well. As manufacturers seek to find replacements, pest managers must use existing products with discernment.

So Many Products, Too Little Information - Pest managers have been inundated with insecticides reported to be effective against bed bugs. For many of these products, efficacy testing is limited. Even when data are available, results may not reflect resistance levels of populations encountered in the field. Bed bug products are often evaluated using long-maintained laboratory strains that become more susceptible to pesticides over time. Lab-in-A-BagTM can provide specific 'point-of-care' information on the actual infestation to be treated - helping to ensure use of products that are most effective. The approach empowers professionals to draw their own conclusions and prescribe treatment accordingly.

An Aid to IPM - Monitoring bed bug susceptibility aids in judicious use of pesticides and is a core principle of integrated pest management. Lab-in-A-Bag[™] may also help to slow resistance by furthering the use of more efficacious materials.

TESTING KIT COMPONENTS:

- 1 Cushioned & Insulated carry bag 4
- 2) Modular evaluation dishes
- 3 Interchangeable substrate inserts

(Furniture Fabric, Wood, & Bedding Fabric)

- 4 Hand magnifier
- 5) Specimen collection forceps
- 6 Dust application brush
- Grease pencil
- 8 Cooling pack



DIRECTIONS FOR USE:

Modular stackable evaluation dishes:

The LightsOut Lab-in-A-Bag™ is designed to be used at the time of initial inspection. Determining susceptibility of a bed bug infestation before treatment helps ensure that control measures will be effective. Each kit contains modular stackable evaluation dishes for collection and confinement of bed bugs, and a supply of interchangeable substrates on which they typically reside and pesticides are applied.



Preparing evaluation dishes:

Users will generally want to pre-treat evaluation dishes with candidate insecticides before arriving the first time at the account. To accomplish this, place some substrate inserts (furniture fabric, wood and/or bedding fabric) onto sheets of newspaper, paper toweling, etc. and lightly treat one or both sides of each disc. Treating both sides ensures that bed bugs will be unable to avoid exposure by crawling underneath in the evaluation dish. Liquid insecticides can be diluted as per the label and applied with a hand-held sprayer, plant mister, etc., while aerosols can be dispensed directly from the original container. Insecticides formulated as dusts can be applied to the various substrates using the small brush provided with the kit.

DIRECTIONS FOR USE:

Allow the treated discs to dry before placing them into the evaluation dishes. Each substrate insert has an attached tab to facilitate insertion and removal. Chemical-resistant gloves should be worn when handling treated discs. With longer-lasting insecticides, extra treated discs can be stored for future use in appropriately labeled, sealed plastic bags in a cool place such as an air conditioned office. If no upcoming evaluations are anticipated, it might pay to prepare a new supply of discs each time using fresh material.

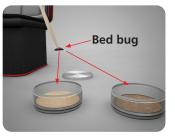


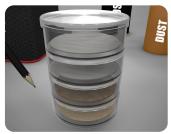


To prepare a stack of evaluation dishes, begin by unscrewing the lid of the topmost dish and insert an untreated substrate disc into the bottom. Maintaining a group of untreated (control) bugs helps to affirm that mortality in the treated dishes is not from mishandling or other causes (see section on **Collecting**, **Handling and Transporting Bed Bugs**). Label this dish with the supplied grease pencil, indicating that no insecticide was applied.

DIRECTIONS FOR USE:







Proceed sequentially down the stack, opening dishes and inserting the insecticides/substrates you wish to evaluate. Pressing discs flush with the bottoms of dishes reduces the chance of bugs crawling beneath the treated substrate (if both sides of discs are pretreated this is less important). To aid in later removal, the pull tab on the substrate disc should be folded up rather beneath the insert.

Remember to label each dish with the product being evaluated, the customer's address/ apartment number, date and time when the sample was collected.

Choosing a Substrate:

Insecticides tend to work better on some substrates than others. This is often influenced by how much toxicant is absorbed versus what remains on the surface to be acquired by pests. Bed bugs tend to congregate on fabric, wood and paper more than metals and plastic - which is why such substrates are included in the kit. The furniture and bedding fabric discs represent substrates that are porous to pesticides, while the wood inserts are less absorptive. When evaluating more than one product, it's probably best to start with a single substrate. Subsequent evaluations can then be made with additional substrates.

Collecting, Handling and Transporting Bed Bugs:

Lab-in-A-Bag™ is designed to be used during initial inspection of a client's property so that decisions can be made about which treatment strategy will be most effective. This requires collecting and transferring bed bugs into evaluation dishes without harming them. The soft-tipped forceps supplied with the kit will help accomplish this. Adults and larger nymphs will be easier to collect than smaller life stages. With a little practice, you'll soon be picking off bed bugs efficiently and without injury. Roughly equal numbers of bugs should be placed into each evaluation dish. Add bed bugs to the topmost (untreated) dish first to avoid contamination. The untreated dish can also serve as a bulk collection reservoir before assigning bugs to individual chambers.

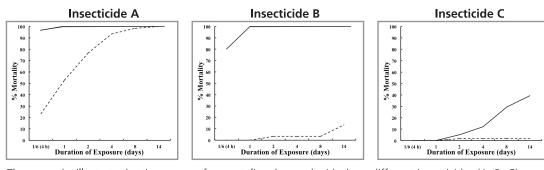
Collecting as many test specimens as possible will increase reliability of the results. A minimum of 4-5 bed bugs per dish is recommended. If only small numbers of bugs can be collected (no more than 10), it may only be possible to evaluate one product, plus the untreated control. With larger infestations, users may want to collect larger quantities of insects for further testing back at the office. A zip-lock plastic bag, containing some crumpled up tissue or paper towel for the insects to cling to works well for this purpose.

Care must be taken to ensure safe passage of collected specimens back to the office. The biggest concern is heat. Transporting bed bugs in a hot service vehicle can result in mortality independent of your insecticide. Lab-In-A-Bag's integrated cold pack and insulated cooler design will aid in the safe transport of specimens. Cold is less of a concern than heat and winter temperatures should seldom cause problems during transit. Upon returning to the office, store the dishes containing bed bugs on a shelf or desktop for subsequent evaluation.

Reading / Interpreting Results:

Readings should be taken daily or as convenient, recording the number of dead bed bugs in each treatment. When in doubt, mortality can be confirmed by opening the dish and gently touching the hind end of the bug with the supplied forceps, pencil, etc. If the bed bug is able to crawl forward it should be considered alive. Survival of bugs in the untreated dish helps ensure that mortality in other dishes is due to the insecticide, rather than other factors.

Some bed bug products are inherently faster-acting than others. Onset and progression of mortality also depends on the resistance level of each population. While susceptible bed bug strains may succumb in a few hours, highly resistant ones may persist much longer with many of the individuals appearing to be unaffected. Such variation in populations is illustrated in the following graphs. Data represent two different bed bug strains (represented by solid and dashed lines) confined on three different insecticides (A, B & C) over a 2-week period.



These graphs illustrate the time course for mortality observed with three different insecticides (A, B, C) on two different bed bug populations. The results are from actual lab tests, and serve to illustrate the variation you may see when exposing different populations to different insecticides. In this example, Insecticide A rapidly kills strain 1 (solid line), but acts more slowly on strain 2 (dashed line). Insecticide B is quick and effective against strain 1, but is ineffective against the other strain. Insecticide C is ineffective against both populations. Either Insecticide A or B would be a good choice against bed bug strain 1, while Insecticide A would be the best for strain 2.

For most insecticides, users should be able to make meaningful determinations within a week, oftentimes in 1-2 days. Some products however tend to be inherently slower acting, requiring somewhat longer evaluation periods.

Cleaning and Care of the Kit:

The evaluations dishes should be cleaned after each complete use. To clean the evaluation dishes use warm soapy water and a soft rag to thoroughly clean each dish. Rinse each dish with warm water until all soap has been removed then leave to fully air dry. Please note that the evaluation dishes are subject to breaking if they are dropped, so please handle with care.

Other Uses for the Kit:

Lab-in-A-Bag[™] has applications beyond selecting products to treat a current customer's bed bug infestation. Because the interchangeable substrates are supplied without pesticide, the kit can be used more broadly to evaluate products of interest, possibly before purchasing bulk quantities In essence, Lab-In-A-Bag[™] empowers the pest management professional to do their own research — independent of those who market and sell to the industry. While insecticide screening trials by universities and independent contractors can be useful, they often use bed bug colonies maintained in the laboratory for a number of years. These tend to be more susceptible to insecticides than populations collected recently from the field.⁵⁻⁶

When large infestations are encountered companies may want to stockpile additional bugs for future evaluations. Maintaining them in a zip lock bag with some crumpled up tissue or paper toweling to cling to will enable them to survive several weeks, if not months. Storing them at cooler temperatures, e.g., 40-65° F will further prolong their survival without having to be fed.

Companies offering heat and other non-chemical methods would benefit from knowing which infestations will be harder to control using pesticides. Firms offering both heat and chemical treatment can use the findings to help tailor their choice of management options for the customer.

Although Lab-in-A-Bag™ was principally designed for bed bugs, the kit can also be used to evaluate products against other pests. Especially those that can be collected in fairly large numbers, and transferred easily into the evaluation dishes (ants, brown marmorated stink bug, Asian lady beetle, etc.).

A Portable, Reusable, Bed Bug Pesticide Resistance Testing Kit



References cited:

- 1. Romero, A., M. F. Potter, D. A. Potter and K. F. Haynes. 2007. Insecticide resistance in the bed bug: a factor in the pest's sudden resurgence? J. Med. Entomol. 44: 175–178.
- 2. Romero, A., M.F. Potter, K.F. Haynes and E. Hardebeck. 2007. Insecticide resistant bed bugs: implications for the industry. Pest Control Technol. 35(7): 42,44,46,48,50,143.
- 3. Zhu, F., J. Wigginton, A. Romero, A. Moore, K. Ferguson, R. Palli, M.F. Potter, K.F. Haynes, and S.R. Palli. 2010. Widespread distribution of knockdown resistance mutations in the bed bug, Cimex lectularius (Hemiptera: Cimicidae), populations in the United States. Arch. Insect Biochem. Phys. 73: 245-57.
- 4. Gordon, J.R., M.H. Goodman, M.F. Potter and K.F. Haynes. 2014. Population variation in and selection for resistance to pyrethroid-neonicotinoid insecticides in the bed bug. Scientific Reports. 4:3836 DOI: 10.1038/smp03836
- 5. Gordon, J.R., M.H. Goodman, M.F. Potter and K.F. Haynes. 2014. Trouble brewing for bed bug insecticides? Pest Control Technol. 42(6): 72-74, 76,78,80.
- 6. Adelman, Z.N., K.A. Kilcullen, R. Koganemaru, R. Anderson, M.A.E. Anderson, T.D. and D.M. Miller. 2011. Deep sequencing of pyrethroid-resistant bed bugs reveals multiple mechanisms of resistance within a single population. PLoS ONE 6: e26228
- 7. Mamidala, P., S.C. Jones, and O. Mittapalli, 2011. Metabolic resistance in bed bugs. Insects. 2, 36–48.
- 8. Zhu, F., H. Gujar, J.R. Gordon, K.F. Haynes, M.F. Potter, and S.R. Palli. 2013. Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. Scientific Reports. 3: 1456 DOI:10.1038/srep01456
- 9. Koganemaru, R., D. M. Miller, and Z.N. Adelman. 2013. Robust cuticular penetration resistance in the common bed bug (Cimex lectularius L.) correlates with increased steady-state transcript levels of CPR-type cuticle protein genes. Pesticide Biochem. Phys. 106: 190-97.