2011

Bed-Bugs.co.uk

Useful information and professional treatment solutions.

Bed Bugs Limited

Prepared by

David Cain

Investigation of Polymerase Chain Reaction based detection of bedbug DNA

Contents

About bedbugs

Bedbugs are blood feeding parasites that preferentially feed on humans. They are a persistent pest and have developed a number of highly evolved abilities to remain close to humans.

They are a pest of exposure and only arrive in your home if you have come into contact with them external to the property or if an adjoining property has a significant infestation.

Close up of an adult bedbug, when fed they become oval in shape but are usually only seen when they are thin and flat.

Close up a juvenile bedbug, the characteristic dark brown colour develops as the bedbug matures and younger samples may appear translucent.

Close up a nesting area showing many of the classic signs of bedbugs, live samples, cast skins and faecal trace signs.

Close up of a bed slat illustrating a build-up of faecal traces and some egg casings close to the joint in the wood.

Bedbugs have been documented as pests since the 17th century although they have been around for much longer and most likely followed man out of the caves millennia ago. Bedbugs were common in the UK prior to World War II, after which time widespread use of synthetic insecticides such as DDT and public education greatly

reduced their numbers, at one stage though in the 1930's 30% of all homes in London were infested.

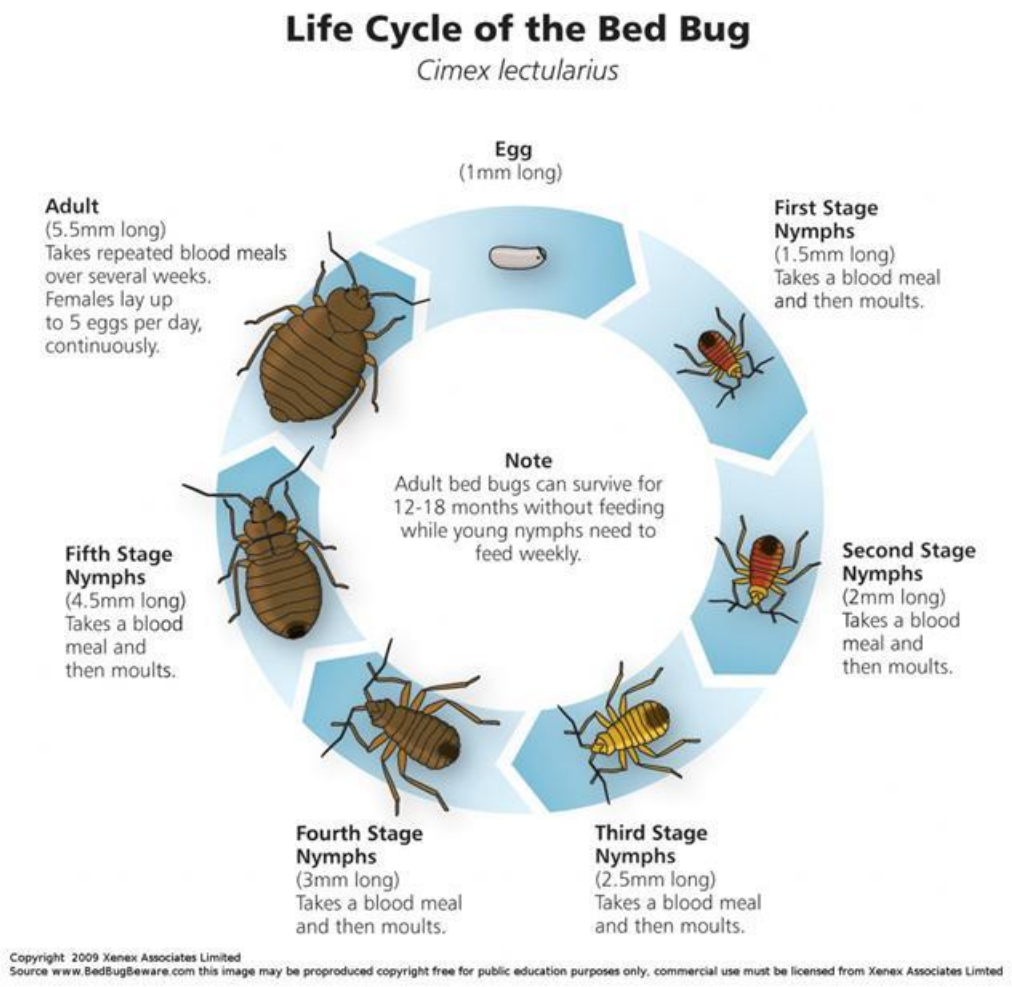
In the past decade, bedbugs have begun making a comeback across the world, although they are not considered to be a major pest or health hazard they can be highly unpleasant to live with and can cause a severe lack of sleep. International travel and commerce are thought to facilitate the spread because eggs, young, and adult bedbugs are readily transported in luggage, clothing, bedding, and furniture. Bedbugs can infest airplanes, ships, trains, and buses, recent cases that we have worked on have been traced back to travel where the source was identified to be the return journey rather than an infested room.

Bedbugs are most frequently found in dwellings with a high rate of occupant turnover, such as hotels, motels, hostels, dormitories, shelters, apartment complexes, tenements, and prisons. Such infestations usually are not a reflection of poor hygiene or bad housekeeping but that a previous occupant had come into contact with them at some stage.

Adult bedbugs are brown to reddish-brown, oval-shaped, flattened, and about 3mm to 5mm long. Their flat shape enables them to readily hide in cracks and crevices. In some cases colonies have been found in places where it is difficult to insert a sheet of paper.

Life Cycle

Female bedbugs lay from 1 to 7 eggs per day, and the eggs are deposited on rough surfaces or in cracks and crevices. The eggs are coated with a sticky substance so they adhere to the substrate. Eggs hatch in around 10 days, and nymphs can immediately begin to feed. They require a blood meal in order to molt and develop into the next stage. Bedbugs reach maturity after five molts. Developmental time (egg to adult) is affected by temperature and takes about 21 days at 30°C to 120 days at 18°C. The nymphal period is greatly prolonged when food is scarce. The adult's lifespan may encompass 12-18 months and they are known to be able to survive for 12 months between feeds although if a source of food is present they will always be active.



Habits

Bedbugs are fast moving insects that tend to be most active at night when we rest; they feed on blood using a piercing mouth part the entry of which is often unnoticed. Nymphs may become engorged with blood within three minutes, whereas a full-grown bedbug usually feeds for ten to fifteen minutes. They then crawl away to a hiding place to digest the meal; a full meal may take 3 or 4 days to digest.

Bedbugs hide during the day in dark protected sites, they prefer fabric, wood, and paper surfaces. They usually occur in fairly close proximity to the host, although they can travel great distances if needed. Bedbugs initially can be found about tufts, seams, and folds of mattresses, later spreading to crevices in the bedstead. In heavier infestations, they also may occupy hiding places further from the bed. They may hide in window and door frames, electrical boxes, floor cracks, baseboards, furniture, and under the tack board of wall-to-wall carpeting. Bedbugs often crawl upward to hide in pictures, wall hangings, drapery pleats, loosened wallpaper, cracks in plaster, and ceiling mouldings.

Injury

The bite is painless at the time but can cause the skin to become irritated and inflamed. Individuals differ greatly in both the extent and timing of their response to a bite. A small, hard, swollen, white welt may develop at the site of each bite which can occur in rows or batches of three or four but also in single reactions. This is often accompanied by severe itching that lasts for several hours to days, in rare cases an allergic reaction may follow, in such cases seek medical attention immediately. The morphology or bites is highly variable and it is almost impossible to diagnose on bites alone.

It is believed that 1 in 10 people show no signs of biting, often leading to the myth that they only attack certain people and about 60% of people do not appear to show signs at the start of an infestation. Cases of extreme reaction seem to be on the increase and affect as many as 2 in 10 people. Given the extent of some of the documented infestation in commercial properties it is clear that waiting for bites to indicate an issue is too unreliable and results in infestations which progress beyond simple and fast control.

Confirming signs

There are only three easily confirmed signs of bedbugs, these are:

- Live samples – although cryptic in nature and small at the nymphal stage they are detectable by those with good eye sight.

- Cast Skins – due to the incomplete metamorphic life cycle of bedbugs they must shed skins between blood meals to develop. This can be a good indication of how long an infestation is present.

- Faecal traces – as bedbugs must defecate after a blood meal and often just before entering a refugia these are the most indicative sign of their presence and can be a good indicator of their locations.

The following are considered to be non-confirming signs:

- Bites – this is because not everyone initially responds to the bites of bedbugs, this fact explains why a hotel can have an undetected infestation for so long and why screening for early detection is such an essential step in an integrated bedbug management systems.

- Blood spots on sheets – an equally variable sign not only due to the different types of blood spots but also due to the fact that not everyone continues to bleed from the puncture wound.

Prevention

In the case of domestic settings prevention can only be achieved through avoidance of this pest. As a pest of exposure bedbugs must always be brought into the home

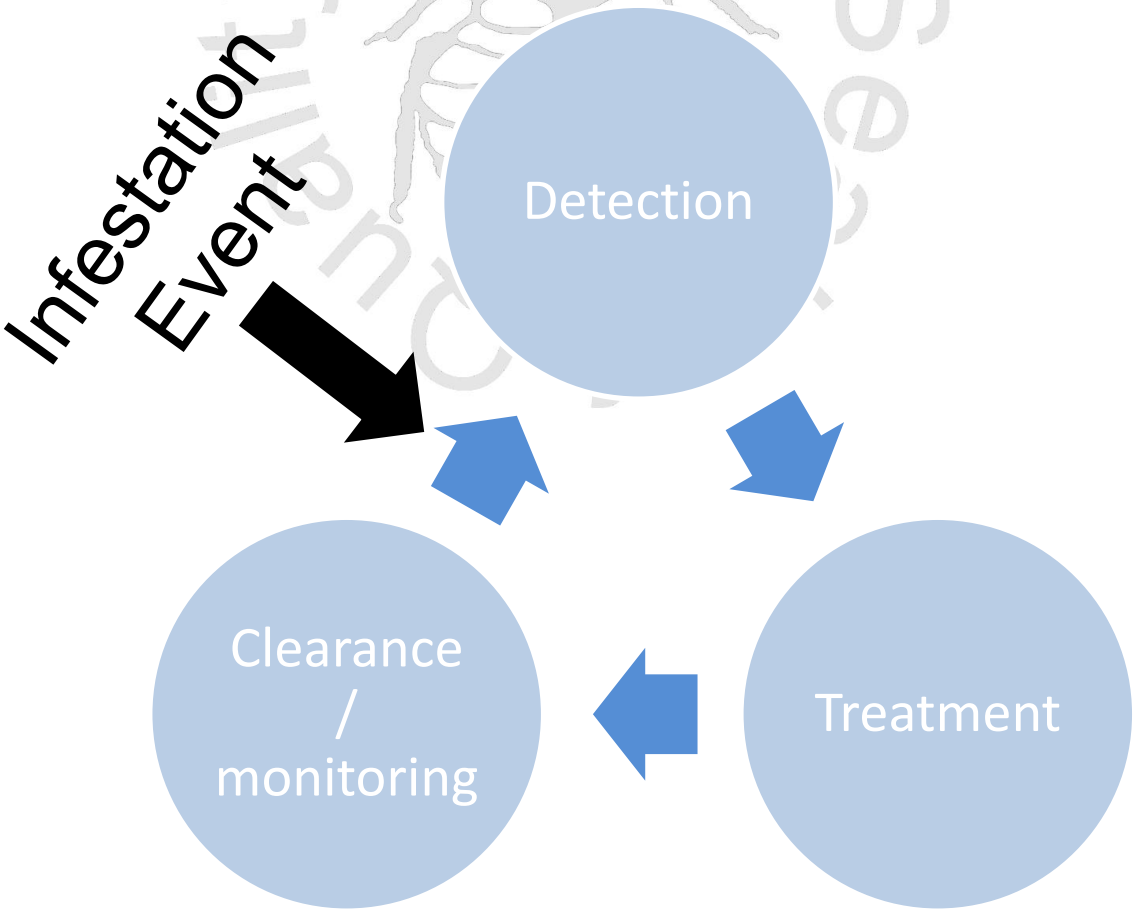
through an introduction event or increasingly through adjoining walls from a neighbouring property. Sources of bedbugs in domestic settings have been traced back to:

- Work locations
- Hotel stays
- Hospital stays
- Public transport
- Second hand items
- Delivered with new items

Domestic prevention is only possible through public education and increased awareness of the need for early detection and avoidance. Although this is a slow process in today's fast media culture it was the only solution in the past and remains an extensively unmet need.

The only truly preventative technology to assist people in home presentation is the PackTite decontamination system (www.PackTite.com) which enables people to heat treat items before they are brought into the home. Although not globally available at present it is a valuable aid to anyone facing a bedbug situation.

The infestation cycle



The infestation cycle of bedbugs encompasses three distinct phases:

- Detection – the first stage of the control cycle is to identify and confirm the issue. Although there are multiple approached to detection the key to an effective system is cost and operational efficiency. Regardless of what method is used the aim should always be to detect the infestation within the first 30 days when it is relatively contained and less likely to have spread through to other rooms.

- Treatment – although this is the part of the cycle which we tend to focus on the most it is in fact clearly the wrong stage for initial focus. The extent of an infestation and therefore the appropriate methods to apply is very much dictated by the extent and duration of the infestation.

- Clearance – in essence a control stage to ensure that the treatment has been effective and that all bedbugs have been eradicated.

The infestation event cannot be modelled or predicted, the only control we can exercise over it is to reduce the period of time between the infestation event and the detection and subsequent control phases of an infestation. The shorter the time between the infestation event and the detection of an infestation the faster the issue can be resolved.

For further information on bedbugs please see the following web resources:

www.Bed-Bugs.co.uk
www.BedBugBeware.com
www.BedBugger.com

Polymerase Chain Reaction

PCR is a molecular biology technique for the amplification of target DNA, it is the building block of DNA finger printing and has revolutionised many areas of research and diagnostics.

A reaction mixture containing amplification primers, free nucleotides, buffers and enzyme are added to the sample in question which is placed in a thermo cycler. The perusing rapid cycling has three steps, denaturation, annealing of primers and extension of primers.

So long as the reaction is working efficiently and the target DNA for the primer set is present the reaction will produce short fragments of DNA which can be visualised or confirmed using a variety of different methods although visualisation by gel electrophoresis is still the most widely used application.

PCR is a widely accepted application for DNA detection although for confirmation of the exact nature of the amplified product DNA sequencing is still the gold standard as it allows full confirmation of the DNA sequence rather than confirmation of a fragment of the correct target size.

Experimental approach

To ensure there was no experimental bias Richard Naylor at Sheffield University in the UK was asked to provide 5 swabbed samples for DNA testing. The only specification was that the samples must include a single negative control with no exposure to bedbug DNA and a variety of other species to ensure the specificity of the testing.

The samples were received at out London facility and posted out to a service company in the US who forwarded them to Research Associates Lab in Dallas Texas for analysis.

Once the results were returned via email they were sent to Richard Naylor so that he could reveal which results matched with the samples that were submitted.

Results

The returned result sheets have been included in this report on the following pages.

Sample number	Species	Reported Result	Pass or fail
1	Tropical bedbug – Cimex hemipterus	Cimex Lectularius (Bedbug) Positive HOT!	Fail
2	Common bedbug – Cimex lectularius	Cimex Lectularius (Bedbug) Positive HOT!	Pass
3	Swallow bug - Oeciacus vicarius	Cimex Lectularius (Bedbug) Positive	Fail
4	Control – no DNA	Cimex Lectularius (Bedbug) Negative	Pass
5	Aphid - Aphidoidea	Cimex Lectularius (Bedbug) Positive	Fail

Research Associates Laboratory
(R.A.L.,Inc.)

14556 Midway Road, Dallas, TX 75244
Phone: (972)960-2221 Fax: (972)960-1997
www.vetdna.com

TEST RESULTS

Acct ID: **P155**
Stern Enviromental Group
Attn: Douglas Stern
100 Plaza Dr.
Secaucus , NJ 07094
Phone: 201-319-9620
Fax: --
Email: d.stern@mindspring.com

Owner Name: **Stern Enviromental Group**
Lab ID: **113839**
Test Date: **10/06/2011**
Animal Name: **A TOP OF SKIRTING BOARD**
Species: **ENVIRONMENTAL**
Medium: **Swab**

Test Description	Result	Comments
Cimex Lectularius (Bedbug)	**Positive**	**HOT!**

NEW TESTS NOW AVAILABLE

PDD - AVIAN BORNAVIRUS
RINGWORM - ALL SPECIES
MICROBIOLOGY - C&S W/ GRAM STAIN

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Attn: Douglas Stern
100 Plaza Dr.
Secaucus , NJ 07094
Phone: 201-319-9620
Fax: --
Email: d.stern@mindspring.com

Owner Name: **Stern Enviromental Group**
Lab ID: **113840**
Test Date: **10/06/2011**
Animal Name: **B HEADBOARD BOX ROOM**
Species: **ENVIRONMENTAL**
Medium: **Swab**

Test Description	Result	Comments
Cimex Lectularius (Bedbug)	**Positive**	**HOT!**

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Stern Enviromental Group
Attn: Douglas Stern
100 Plaza Dr.
Secaucus , NJ 07094
Phone: 201-319-9620
Fax: --
Email: d.stern@mindspring.com

Owner Name: **Stern Enviromental Group**
Lab ID: **113841**
Test Date: **10/06/2011**
Animal Name: **C CRUSHED BUG GREENHOW ST.**
Species: **ENVIRONMENTAL**
Medium: **Swab**

Test Description	Result	Comments
Cimex Lectularius (Bedbug)	**Positive**	

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TEST RESULTS

Acct ID:**P155**
Stern Enviromental Group
Attn: Douglas Stern
100 Plaza Dr.
Secaucus , NJ 07094
Phone: 201-319-9620
Fax: --
Email: d.stern@mindspring.com

Owner Name: **Stern Enviromental Group**
Lab ID: **113842**
Test Date: **10/06/2011**
Animal Name: **D BED SHEET MASTERBED**
Species: **ENVIRONMENTAL**
Medium: **Swab**

Test Description	Result	Comments
Cimex Lectularius (Bedbug)	**Negative**	

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Stern Enviromental Group
Attn: Douglas Stern
100 Plaza Dr.
Secaucus , NJ 07094
Phone: 201-319-9620
Fax: --
Email: d.stern@mindspring.com

Owner Name: **Stern Enviromental Group**
Lab ID: **113843**
Test Date: **10/06/2011**
Animal Name: **E KITCHEN, TOWNEND 57**
Species: **ENVIRONMENTAL**
Medium: **Swab**

Test Description	Result	Comments
Cimex Lectularius (Bedbug)	**Positive**	

NEW TESTS NOW AVAILABLE

PDD - AVIAN BORNAVIRUS
RINGWORM - ALL SPECIES
MICROBIOLOGY - C&S W/ GRAM STAIN

If the assay were subject to a contamination we would have had positives in all results, clearly this is not the case as the negative control was clear.

If the sample collection process were contaminated we would expect to see positives in all the samples including the control samples.

Although it is feasible that the lab samples 1 – 3 could have been contaminated as they were taken in the same building this would require an airborne source of DNA which is unlikely to have resulted in the HOT rating of sample 2.

Given that sample 5 the aphid was collecting from the researchers garden the probability of a cross contamination is extremely unlikely and beyond the realms of statistical probability.

Conclusions

Based on the results obtained it is clear that the PCR test is not specific to Cimex lectularius in that it is unable to differentiate between either closely related species such as tropical bedbugs, swallow bugs but also distant relatives such as aphids.

Given this lack to specificity alone it is reasonable to conclude that the amplification primers used are not specific enough for the test performed, although the sample set was not large enough to draw statistically significant results it does indicate the need for further testing to confirm the validity of this and other services.

At this stage without improvements to the accuracy of the science this type of service should not be recommended to consumers as clearly the claims made in publicising the service are not supported by the data we have seen.

In researching this service we also came into possession of documents that indicate that many of these flaws and limitations may have already been brought to light in some circles and yet had not been passed on to consumers.

This again highlights the need for the pest management industry to have a consumer centric standard to test all products and services against which must by definition be free from the influence of manufactures and the distribution chain. Without this being put in place it will become increasingly difficult for consumers to remain confident in professional solutions and services.

Suggested applications of molecular techniques

Although in this instance we have proven that there is no value to the results of this DNA testing service for bedbugs we cannot dismiss the value that could be provided through the use of molecular diagnostic tools in combating bedbugs. In particular it is technically feasible to use molecular techniques to:

- Genotype field samples to identify any issues relating to genetic tolerance or resistance to insecticide to aid product selection.

- To genotype samples to identify common sources within building or local areas to help identify and isolate "local" source infestations.
- Species identification through the use of species specific amplification primers rather than the set that is currently being used which is essential given the differences in treatment patterns for species other than Cimex lectularius and hemipterous.

Some of this could be conducted with mutation specific amplification primers in a multiplex PCR reaction although the time and equipment needed for amplification means that it is unlikely to ever be a field application for routine pest control. It would however provide valuable information should local or regional universities start offering this level of collaborative service for their local pest controllers and bedbug specialists in conjunction with public health departments.

Acknowledgements

The author of this report would like to thank the following individuals for their support in conducting this project:

Richard Naylor – Entomologist and bedbug biology specialist at Sheffield University UK for conducting the double blind sample collection under scientific and sterile conditions and for kind permission to use one of the images in this report.

Douglas Stern- Stern Environmental New Jersey USA for submitted the samples anonymously and ensuring that they were treated as a routine customers sample set and were not given special attention by the lab.

The readers and contributors of bedbugger.com/forum for being concerned enough to encourage me to fund this project on behalf of consumers the world over.

About the author

David Cain has been on a tireless quest to take on bedbugs since first exposed to them in 2002 when he finished a career in science and business to peruse a different direction in life. Having qualified as a molecular biologist from Nottingham and Leicester Universities in the UK he worked in various laboratory and technical roles until eventually working for investment banks and venture capitalists assessing the value and investment risks of scientific based business models.

In 2005 Bed-Bugs.co.uk was started as the world first dedicated extermination firm working exclusively on bedbugs since the demise of Tiffin & Son who operated between 1650 and the 1930's.

Passionate about bedbugs and how the industry is developing on a global basis he spends an excessive amount of time trying to help people and keep the information in the public domain accurate and helpful.

Appendix

In order to better understand what could have gone wrong I gave the service provider a chance to explain. The email trail is included below:

Submitted request for comment

Below is the result of your feedback form. It was submitted by (dcain@bed-bugs.co.uk) on Friday, October 14, 2011 at 16:19:07
---

firstname: David

lastname: Cain

Description: Other

Question: Hi,

> I recently submitted some samples for bedbug detection as a test of the service. They came back as 4 positive for bedbugs Cimex Lectularius and 1 negative.
>
> Only 1 of the samples was in fact Cimex Lectularius with the others being 1 negative control (correct identified) and 3 other species of insects (incorrectly identified).
>
> Could you please confirm if the primer sets you are using for amplification are species specific to Cimex Lecularius and if so why I would get such an inaccurate result.
>
> I am preparing a research paper on the test at present and will be publishing it early next week.
>
> Thanks in advance.
>
> David Cain

Submit: Submit

Reply

Hi David,
Thank you for your questions. Our Bed Bug DNA test detects Cimex lectularius only. It does not detect other insects or other Cimex species. Sample contamination is the most obvious problem. Many people accidentally contaminate samples by not properly handling them or following proper scientific principles. If 4 of the samples tested positive, it was due to the fact that Bed Bug DNA was present on the samples submitted. Our test does not have false positives and proper scientific controls are in place within our facility to prevent such

confusion. We do have a separate DNA test that will detect the other Cimex species, but is only used when specifically requested.
I would hope one would be more thorough with real information and research before considering this a publishable paper and worthy of presentation anywhere. The errors, if any are certainly on your end. At the recent Bed Bug symposium you purposely avoided us for discussions, we even had a booth present containing two of our scientists. Your obvious lack of understanding both the art of PCR and your inabilities combine to make you nothing more than a nuisance. I have personally performed over 100,000 Real-Time PCR tests and find your recent on-line comments both offensive and ignorant. If you have a peer group that finds your 'research' credible then please present my comments to them. Any further contact from your emails will find their way to our spam blocker, where they belong.

Sincerely,
Ernie

Ernest Colaizzi, President
Research Associates Laboratory, Inc,
14556 Midway Road
Dallas, Texas
972-960-2221
972-960-1997 fax
214-686-2026 cell
rallab@aol.com
www.vetdna.com

Response

Hi Emie,

To prevent the risk of any contamination the samples were generated by an entomologist in an independent lab using sterile techniques. I am not suspecting a cross contamination in your processes as the negative control would have been positive.

I suspect that the amplification primers are not specific to Cimex lectularius as they also amplified Cimex hemipterous as well as one other closely related species and one more distant species.

I will include this email communication in the research report as I think it is now a relevant document.

I am sorry if you do not get a chance to read this and reply because you have put us in the spam filter, I can assure you I will not lose any sleep. For the record I hold two degrees in molecular biology from Nottingham and Leicester Universities in the UK.

Regards,

David Cain

Disclaimer

This test was funded by Bed Bugs Limited in London UK on behalf of concerned consumers to ensure that the claims made by the detection lab were in line with their labs ability to conduct and interpret DNA analysis on samples.

Although we have developed bedbug detection technology it is not in this class and is only mentioned for the avoidance of doubt that there is no conflict of interest between this approach to DNA testing and the current or future services of the company.