Sample Collection Protocol for HSC Submission

The following section outlines activities relating to the collection, handling and analysis of dust samples collected from visited homes enrolled in this research project. Relevant sections for this research project are adapted from the National Survey of Lead Hazards and Allergens in Housing: Protocol and Sample Design Report Revision 4 that was prepared for the Office of Lead Hazard Control, U.S. Department of Housing and Urban development and the National Institute of Environmental Health Sciences. Sampling methods and techniques are adapted from the National Survey of Lead and Allergens in Housing.

Dust samples will be collected via the *Allergen Dust Vacuum Method* described herein and subsequently analyzed for: dust mite, cockroach, and alternaria**.** During the home visit, a trained team including the *Interviewer* and *Technician* will follow standardized protocols to perform the sampling, as well as other evaluations and inspections as outlined by the study protocol.

Personnel Training and Standardization

Interviewers and technicians will undergo a standardized training program prior to beginning home visitations and sampling. A *Sampling Handbook* will be created outlining all of the information necessary to conduct successful field sampling of each housing unit (HU). Training will cover areas that include:

1. Overview of protocol and purpose
2. Interviewing skills and code of conduct in homes
3. Orientation to data collection forms and appropriate completion
4. Orientation to sampling devices
5. Handling of sampling materials
6. Handling and transport of collected samples (valid and invalid samples)
7. Troubleshooting of likely encountered problems

Interviewers and technicians must satisfactorily display proficiencies in the areas described above prior to being sent to the field. Copies of the *Sampling Handbook* will be given to each interviewer and technician team.

Vacuum Sampling Materials and Supplies

Each team will be supplied with the following items in order to conduct their dust sampling:

1. Eureka Might Mite® vacuum

   This is a HEPA (High Efficiency Particulate Air) sealed vacuum filtration system, which exhausts all vacuumed air including the air from the motor through a HEPA filter. This process reduces to a minimum the amount of allergen dispersed into the air. The vent on the vacuum will remain in the closed position to allow for the greatest suction force.

2. Supply of crevice tools
   Four crevice tools will be supplied for each sampled housing unit, and a crevice tool bag to place all used crevice tools for return to the coordinating center office for cleaning and reuse.
3. Vacuum filter bags (extra and replaced every 3 months)
4. Extension cord (25 feet) with 2-prong adapter
5. Box of disposable wipes for cleaning hands and sampling tools
6. Non-sterilized, non-powdered disposable gloves
7. Ziploc® baggies
8. Surgical booties
9. Timer
10. Temperature/relative humidity gauge
11. Allergen dust sample logs and labels
    There will be 4 different Allergen Dust Sample logs, one for each sample collected. The interviewer and/or technician will complete these logs.
12. Template: folding frame to create a 1 square meter template
13. Cooler with cold packs to keep samples refrigerated during transport
    Dry cold packs will be used. Ice packs may generate moisture and leak, ruining the samples.
14. Trash bags
    No trash generated by the sampling will remain in the HU. All trash will be placed in these bags and disposed of properly off-site.

Dust Sample Collection Sites

Allergen dust samples will be collected from the floor in each of the specified rooms. These rooms have been selected by guidelines established by the National Survey of Lead and Allergens in Housing document published by HUD. See Table S1 for details of sampled rooms. In addition to floor samples, an allergen vacuum sample will also be collected from the bedding in the enrolled child's bedroom.

The rooms that will be sampled include:

1. Kitchen
   The floor dust sample will be collected from the entire kitchen. First along the base of all cabinets as well as between the refrigerator and stove and cabinets or walls, then the general floor area until 5 minutes sampling time has been completed.

2. Common living area (living room, den, family room, etc.)
   For both this room and the bedroom, floor dust samples will be collected from 2 square meters directly adjacent to the sofa and bed respectively. The allergen floor dust samples will be collected before the bedding samples. Upholstered furniture will not be sampled.

3. Bedroom
   Bedrooms are rooms that people sleep in more than 6 nights per week. Rooms that were designed as bedrooms, but are being used for another

purpose, e.g. as a guestroom, office, playroom, sewing room, or storage room, are not included as bedrooms. Only the bedroom of the enrolled child will be sampled. Occasionally, a bed may not be a conventional bed (i.e. it may be a couch or a pad) these will be sampled in a manner similar to a conventional bed.

If possible, 0.25 square meter of the area (approximately 10 inches by 39inches) will be vacuumed under the child's bed. If this is not possible (i.e. because the mattress is on the floor, or objects under the bed prevent access), as much of the 0.25 square meter area will be vacuumed as possible. If there is a small area rug in the area by the bed, it will be vacuumed as if it were a smooth surface.

The bedding sample will be collected in the pre-selected bedroom from the bed most often slept in by the enrolled child. All bedding layers will be handled with care as to not get stepped on. Occupants may be asked to assist in the sampling of the bedding. All layers of the bedding will be vacuumed (i.e. Covers, blankets, top sheets, bottom sheet, mattress pad, "egg-carton" style pads, mattress and pillows) for a total of 5 minutes. Two square meters of the bedding will be vacuumed: if the bed is a single bed, the entire surface; if it is a double bed or larger, one meter width will be measured and vacuumed down the length of the bed within the 1 meter wide area. The breakdown of the bedding sample follows:

1. 30 seconds—one pillow (preferable the primary sleeping pillow) inside the pillowcase (if present) without removing the pillowcase, and both sides of the pillow.
2. 2.5 minutes—all of the bedding layers described above.
3. 2 minutes—mattress surface (impermeable, fully encapsulated mattresses will be sampled by vacuuming the top layer. The cover will not be removed).

The following items will not be vacuumed: stuffed animals, under the mattress, towels, and box spring surfaces. Rolled up blankets (that act as pillows) will be vacuumed if they are rolled up and on the bed at the time of sampling.

Table S1. General Dust Sampling Strategy within Each Selected Housing Unit

Rooms/Area	Surface Tested in Each Room/Area	Number of Samples per Room	Sample Location
Kitchen			
Allergen	Floor	1	Entire floor surface (including behind fridge and stove) area is measured
Common Living Area			
Allergen	Floor	1	Front of sofa/chair up to 2 square meters
Bedroom			
Allergen	Floor Bed	1 1	Side of bed up to 2 sq meter All bedding layers

General Sampling Procedures

1. Upon entering the room to be sampled, the chosen area will be determined and walking on the area will be avoided.
2. Vacuum will be plugged in and the cord length will be assured to be long enough to reach the area. The vacuum will not be plugged into a circuit believed to be supplying electricity to an air conditioner, water heater. This will avoid overloading and tripping of the breaker or blowing of the fuse. Teams will be allowed to reset breakers only if such an event would occur. The team can assist the occupant in the usual procedure to rectify the event (contact building manager, landlord, etc.)
3. A new MiteTest collector nozzle/filter thimble will be used at each sampling site (the filter thimble will already be inserted into the collector nozzle in the lab to avoid field contamination). Trash generated will be disposed of in supplied trash bags.
4. Handling of sampling bags will occur only when wearing latex gloves.
5. Vacuuming of the specified areas will begin
6. Surgical booties will be worn during the vacuum process
7. Once sampling is completed the collector nozzle with filter thimble will remain assembled and placed carefully into the original Zip-Loc bag.
8. The filter thimble will be removed and packaged upon returning to the laboratory.
9. The used collector nozzle will be cleaned using a contact cleaner (ie. Contrad 70)
10. Label sample with appropriate code numbers (location of sample, date of sample, interviewer code number and subject code number)
11. Place sealed bag into cooler
12. Complete data collection forms for each respective room.
13. Back at the laboratory, a Chain of Custody form is completed to indicate the source of each sample set, the surface characteristics at the time of sampling and area involved in sampling.

Below is information regarding Contrad 70 obtained from the following website on 1/21/2004: http://www.deconlabs.com/cleaning/contrad70.htm

CONTRAD® 70 cleans any surface...perfectly! And it rinses away totally, leaving no detectable residues. It's phosphate-free, chlorine-free and disposable straight to the drain.

CONTRAD® 70 works by soaking alone, there's no need to scrub. Use it static or in an ultrasonic tank, hot or cold. Create a working solution to remove even the most difficult contaminants from glass, plastics, ceramics and metals.

From routine labware washing to full-scale biotech plant cleaning - when the standard you demand allows no compromise - specify **CONTRAD® clean!**

A few of the many benefits of using CONTRAD® 70

- Cleans everything perfectly
- Totally rinsable, below monomolecular layer level
- Biodegradable, disposable directly to drain
- Replaces solvents, acids and caustics, much safer to use
- Phosphate-free, chlorine-free
- Totally effective radioactive decontaminant
  Satisfies all your cleaning needs!

Typical uses

- Environmental labs use CONTRAD® 70 because it is phosphate-free and leaves no residue to interfere with analytical techniques
- CONTRAD® 70 cleans fermentors, mixers, filling and production equipment in biotechnology and pharmaceutical plants
- Industrial and research labs use CONTRAD® 70 for cleaning glassware, plastics, metals and ceramics
- CONTRAD® 70 is USDA approved for cleaning food and drink process plants
- Clinical labs use CONTRAD® 70 to soak away blood and proteins on critical analytical labware
- CONTRAD® 70 works for you...ultrasonics, radioactive decontamination, flow-through cleaning, CIP systems... **Soak it and see!**