（✓Abroad・Domestic）Internships report form（Student） 2014/05/21 (Year/Month/Day)

Name	Nzelu Chukwunonso Onyemaechi
Laboratory	Parasitology
Year (Grade)	D3
Internship institution	National Institute of Allergy and Infectious Diseases, NIH, USA
Internship period	Feb, 15, 2014 - May 14, 2014
Purpose	1. Training on establishment and maintenance of sand fly colonies. 2. Research on genetic exchange during cyclical development of *Leishmania* in the sand fly vectors.

Summary of activities （about 800 words、provide photos, tables and figures that clearly show the activities during the period）

Establishment and maintenance of sand fly colonies

I was trained on how to initiate new sand fly colonies, and maintenance of laboratory colonies.

For the initiation of colonies, sand flies immobilized by cold are sorted according to sex, and blood-fed females intended for the establishment of colonies are selected and transferred into plaster-lined plastic pots with screened lid (Fig. 1a). The pots are kept in plastic rectangular boxes with tight-fitting lids. High humidity is ensured by placing moistened foam in the box. Females are provided with a 30% sucrose solution and kept at 25 – 26^{O}C and afterwards pots are soaked in water to enhance egg laying. After oviposition, the eggs are washed with distilled water and set up for the full rearing of the colonies (fig. 1b). Larvae hatch from eggs usually after six to ten days. Larval food is a composted mixture of rabbit faeces and food (fig. 1d). The larval period (four instars) usually lasts for three weeks or more depending on the species, followed by pupation period. Emerged adults are released from pots into cages and both sexes fed on sugar solutions (fig 1c). Later, females are offered a blood meal on anesthetized mice. Fed females are left undisturbed in cages for 48 h and maintained on sugar solution. On the next day, the fed females are transferred into oviposition pots for generation of progenies.

Rearing of colonies (routine maintenance) and preparation of larval food in the laboratory

I received training on how to maintain different colonies of sand flies: *Phlebotomus* and *Lutzomyia* species. All the colonies were maintained in an incubator at 25 to 26^{O}C in the insectary (fig 1e). During the maintenance training on the full life cycle of each of the colony, I was able to compare major life cycle parameters between various colonies and understood their different standard laboratory conditions. At the course of the training, I learnt how to prepare larval food and other equipments used for the maintenance of sand fly colonies in the

insectary.

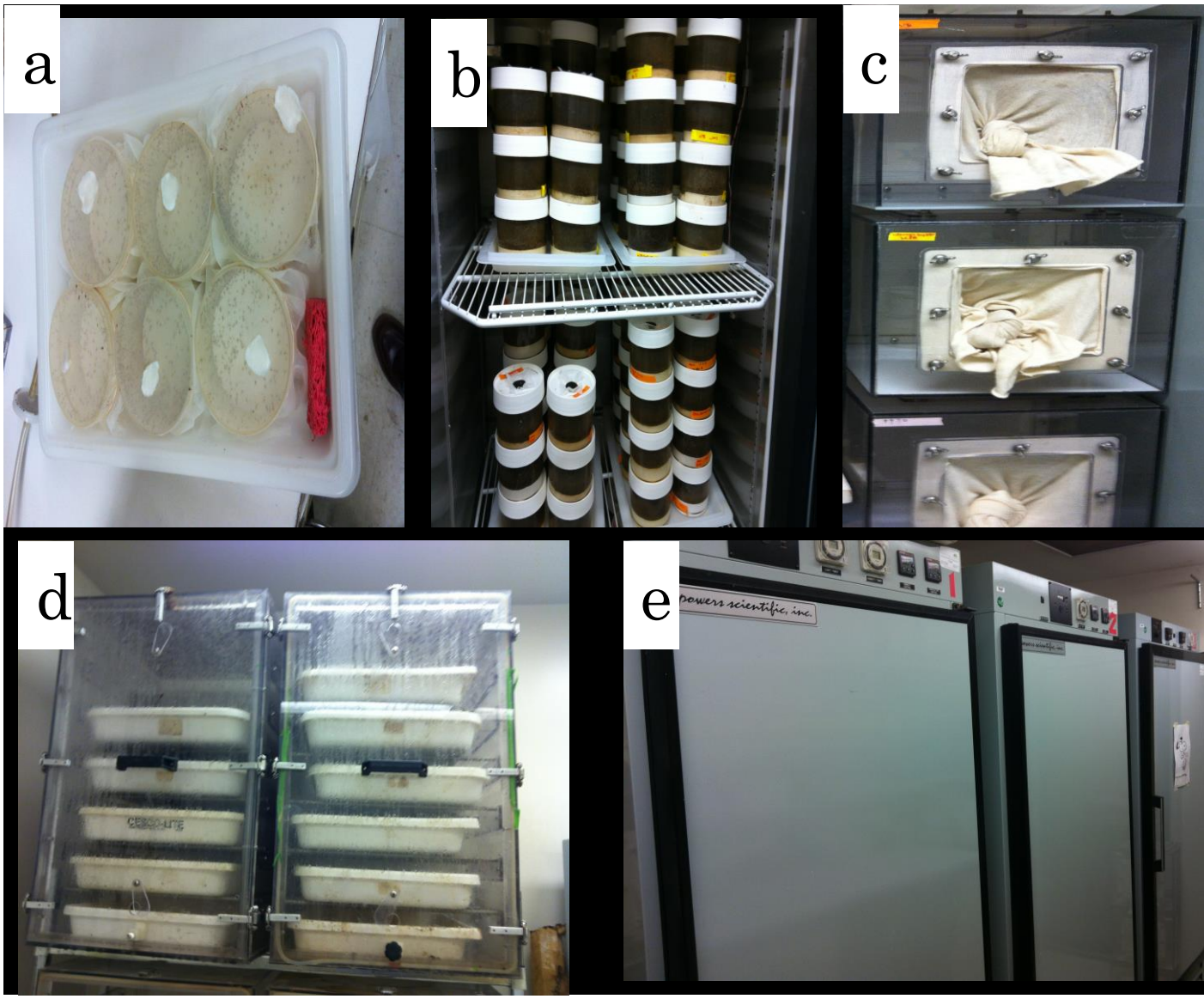

Fig 1. Some of the equipments used for sand fly maintenance. a: blood-fed female pots. b: larval pots maintained in incubator. c: adult cages for different colonies. d: two cabinets for fermentation of larval food. e: incubator for maintenance of phlebotomine sand fly colonies.

Generation and evaluation of genetic parasite hybrids

Research into genetic exchange in *Leishmania* parasites has been the focus of the laboratory I visited during my externship at the NIAID/NIH, MD USA. The laboratory was the first to demonstrate that the invertebrate stages of *Leishmania* are capable of having a sexual cycle consistent with a meiotic process. As part of my

training, I was involved in the experimental crosses in sand flies aimed at generating and evaluating genetic parasite hybrids. My research was to explore the mating competency of the F1 hybrid progeny for generation of backcross, outcross and selfing hybrids or F2 genotypes required for linkage analysis. The study involved manipulation of sand flies for infection, culture of the *Leishmania* parasites, establishment and evaluation of infections in the flies. During the experiments, I carried out several experimental crosses (*Leishmania* infection) in the *Phlebotomus duboscqi* vector through membrane feeding on *Leishmania* infected mouse blood (fig. 2a). Sand fly midguts were dissected at different days post-infection to evaluate the status of infection in these flies.

Following co-infection of flies with parental clone, out clone and F1 hybrides bearing independent drug markers (hygromycin B, nourseothricin and blastocidin resistance cassette), maintained at 26°C, doubly-drug resistant hybrid progeny were selected from the cultures. The frequency of hybrid recovery was calculated as the percentage of the number of clean midgut yielding a doubly-drug resistant population.

In all selected clones, the presence of both parental drug markers was confirmed by Polymerase chain reaction (PCR) tests with primers specific for the parental markers. Additionally, all clonal lines were analyzed for DNA content and genotyping analysis.

* For the results and other details, right belongs to the senior investigator (NIAID/NIH)

Experimental infections with *Leishmania*

I was also trained on the experimental t

ransmission of *Leishmania* parasites to mice by an infected sand fly bite (fig. 2b) and recovery of *Leishmania* parasite from infected mice.

Fig 2. Experimental *Leishmania* infection. a: membrane feeding of flies on *Leishmania* infected mouse blood. b: experimental transmission of *Leishmania* to mice by sand fly bite.

Approval of supervisor	Institution・Official title・Name Graduate school of Veterinary Medicine. Prof. Ken Katakura 印

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