An Efficient Production Process for Extracting Salivary Glands from Mosquitoes

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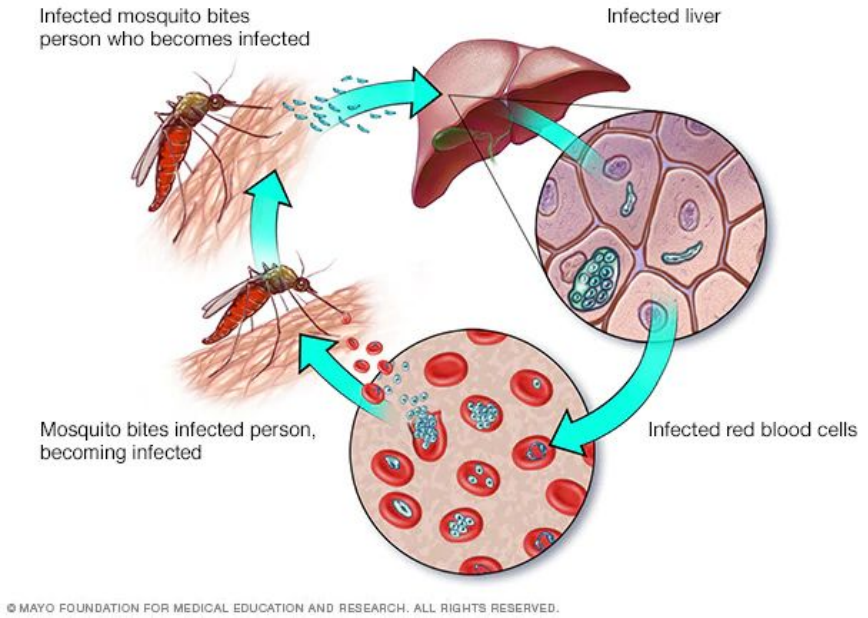
Background

- Sanaria Inc. (Rockville, MD), has developed a clinically effective live malaria vaccine
- Extracting the malaria vaccine from mosquitos is labour and time intensive
- LCSR + Sanaria are developing an automated mosquito dissector to allow for mass production of the vaccine



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Project Summary: Automated vision system

- We aim to use computer vision to guide the automated robotic system
- Uses include mosquito identification, localization of proboscis, localization of head and neck

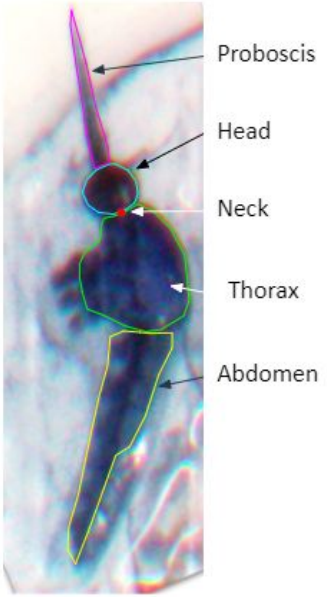

Figure 1. Parts of a mosquito

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Relevance to our Project

- This paper describes the design and evaluation of “semi-autonomous mosquito microdissection system” (SAMMS)
- SAMMS provided the basis for many of the mechanical methods used in the robot today
- Our project & the paper have the same goals
    - Increasing malaria vaccine production through increased automation

“An Efficient Production Process for Extracting Salivary Glands from Mosquitoes”

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Key Results

1. Mosquito dissection was deskilled, reducing training time for new operators by 10-15 fold
2. Mosquitos were dissected in batches instead of individually, increasing throughput

Introduction

- Sanaria developed a clinically effective live vaccine against *Plasmodium falsiparum* sporozoite (PfSPZ), the parasite responsible for malaria
- The parasite resides in mosquito salivary glands; extracting them is labour and time intensive
- Throughput at the time of publication was 300-360 mosquitos/hour, and one lot of vaccine needs at least 50K

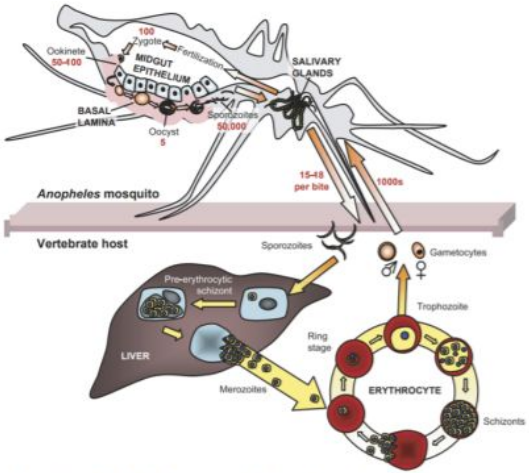

Fig 1. Life cycle of *plasmodium falciparum.* Note the location of the salivary glands in the mosquito. [1]

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Materials & Methods: SAMMS Design

- The authors designed SAMMS to automate difficult parts of mosquito dissection
- SAMMS is comprised of:
  - Staging area
  - Sorting cartridge
  - Automated blade assembly
  - Comb squeezer

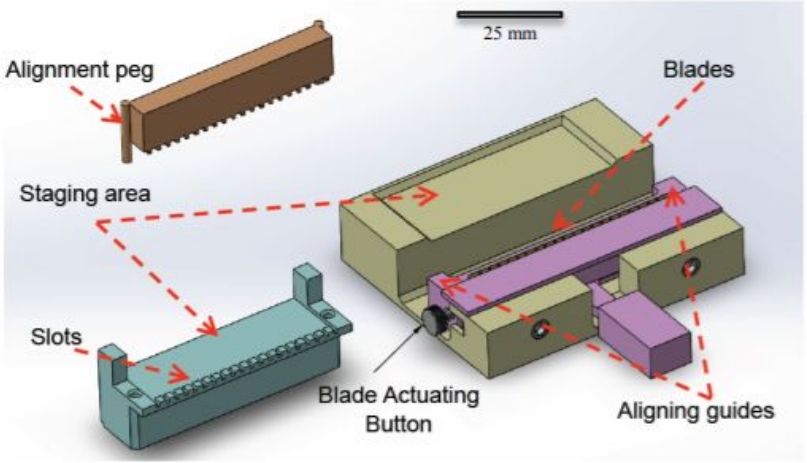

Fig. 3. Design of mosquito gland extraction apparatus, including the sorting cartridge (blue), the blade assembly (pink), squeezer (brown) and staging area (tan).

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Materials & Methods: SAMMS Workflow

1. Place mosquitos on staging area
2. Align 20 mosquitos on sorting comb
3. Actuate blade to decapitate mosquitos
4. Use comb squeezer to squeeze mosquito salivary glands out of thorax
5. Pipette salivary glands for vaccine harvesting

Fig. 4. Decapitation. (Top) Mosquitoes aligned in cartridge with heads between decapitation blades. (Bottom) Mosquitoes after decapitation.

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Materials & Methods: Evaluation

1. Evaluation of mosquito throughput
    a. Mosquitos dissected per hour (Mdph) was evaluated comparing SAMMS to individual mosquito dissection

2. Evaluation of training time for new operators
    a. Training time for mosquito operators to reach proficiency (600 mosquitos/hour) was evaluated for SAMMS vs individual mosquito dissection

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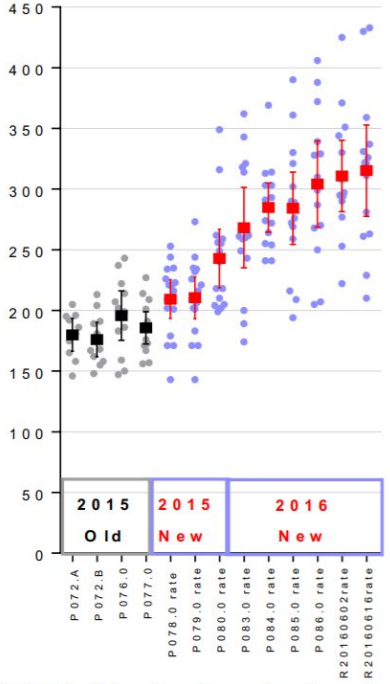
Results: Increase in mosquito throughput

- Shown left are average recorded mosquitos dissected per hour on a given day for a skilled technician
- Mosquito throughput using the SAMMS system is increased compared to the old system
  - 2015 old, black bars/grey dots are the old system
  - 2015 new/2016 new, blue bars/red dots are SAMMS



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Results: Decrease in necessary training time

- Training time for new operators to reach proficiency (600 mosquitos/hour) reduced from 29 weeks on average to 1-3 days
- This indicates a 10-15 fold reduction in training time for new operators

Table 2: Operation times and production rates for 8 operators using a proposed apparatus. **(A)** Minutes to align 20 mosquitoes; **(B)** Minutes for gland extrusion and collection. Total time for 20 mosquitoes is in minutes, and rate is mosquito throughput per hour.

	1	2	3	4	5	6	7	8	Avg
(A)	2.4	2.5	2.3	2.5	1.5	1.3	1.1	1.2	**1.9**
(B)	0.7	0.8	0.5	1.1	1.2	0.7	1.1	0.7	**0.8**
Total	3.1	3.3	2.8	3.6	2.7	2	2.2	1.9	**2.7**
Rate	393	364	429	338	444	600	545	649	**470**

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Discussion

- SAMMS was able to reduce training time and increase throughput compared to single mosquito dissections
- SAMMS is scalable to a larger number of mosquitos, authors believe optimum is 40
- Yield from SAMMS was similar or better than manual dissection
- Future steps lie in vision guided autonomous robot for dissection

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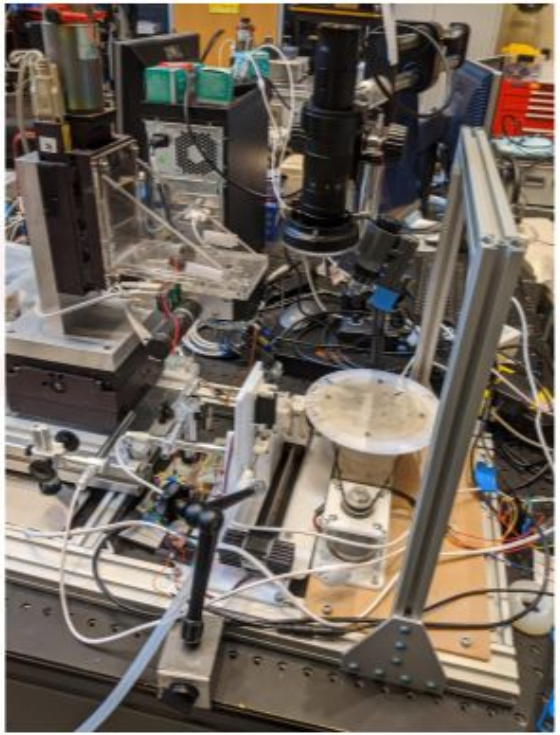
Next Steps

1. Implement a cGMP compliant version of SAMMS for FDA phase III clinical trials
2. Implement a vision-guided fully autonomous robotic mosquito dissector
    a. Development of a robotic implementation of SAMMS
    b. Development of vision algorithms to assist robotic guidance



Strengths & Limitations

Strengths

1. SAMMS is a great innovation; it has a simple design but robust performance
2. SAMMS is also scalable, allowing for even greater throughput
3. Metrics the paper uses (throughput and training time) are simple and relatable to end goal

Limitations

1. More information about shortcomings would be useful (i.e. rate limiting factors)
2. Hard numbers on yield would be better than a statement in the discussion
3. Reasons for vision-guided system alluded to but reasoning not clearly communicated

References

1. **M. Schrum, A. Canezin, S. Chakravarty, M. Laskowski, S. Comert, Y. Sevimli, G. S. Chirikjian, Stephen L. Hoffman, and R. H. Taylor, "An Efficient Production Process for Extracting Salivary Glands from Mosquitoes", arXIV, 2019, http://arxiv.org/abs/1903.02532.**
2. H. Phalen, P. Vagdargi, M. Pozin, S. Chakravarty, G. S. Chirikjian, I. Iordachita, and R. H. Taylor, "Mosquito Pick-and-Place: Automating a Key Step in PfSPZ-based Malaria Vaccine Production", in IEEE Conference on Automation Science and Engineering (CASE), Vancouver, BC, August 22-26, 2019. pp. 12-17.
3. H. Wu, J. Mu, T. Da, M. Xu, R. H. Taylor, I. Iordachita, and G. S. Chirikjian, "Multi-mosquito object detection and 2D pose estimation for automation of PfSPZ malaria vaccine production", in IEEE 15th International Conference on Automation Science and Engineering (CASE), Vancouver, BC, August 22-26, 2019.
4. M. Xu, S. Lyu, Y. Xu, C. Kocabalkanli, B. K. Chirikjian, J. S. Chirikjian, J. Davis, J. S. Kim, I. Iordachita, R. H. Taylor, and G. S. Chirikjian, "Mosquito Staging Apparatus for producing PfSPZ Malaria Vaccines", in IEEE 15th International Conference on Automation Science and Engineering (CASE), Vancouver, BC, August 22-26, 2019.