

1 **A novel restraint device for injection of *Galleria mellonella* larvae that minimises the**
2 **risk of accidental operator needle stick injury**

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16 Staphylococcus aureus; restraint; injection; needle stick injury; *Galleria* Grabber

17 **Abstract**

18 Larvae of the insect *Galleria mellonella* are increasingly being used for studying pathogenic
19 microbes and their virulence mechanisms, and as a rapid model for screening novel
20 antimicrobial agents. The larvae (waxworms) are most frequently infected by injection of
21 pathogenic organisms into the haemocoel through the insect's prolegs. The mostly widely
22 used method for restraining the waxworms for injection is by grasping them between the
23 operator's fingers, which puts the operator at risk of needle stick injury, an important
24 consideration when working with highly pathogenic and/or drug-resistant microorganisms.
25 While use of a stab proof glove can reduce this risk of injury, it does so at the loss of manual
26 dexterity and speed, resulting in a more labour-intensive and cumbersome assay. We describe
27 a simple cost effective device (the so-called '*Galleria* Grabber') for restraining waxworms
28 for injection that keeps the operator's fingers clear of the needle thus reducing the risk of
29 injury.

30

31 **Introduction**

32 Larvae (waxworms) of the Greater wax moth *Galleria melonella* have become a widely used
33 surrogate host for studying pathogenic microbes. In recent years, they have been used for
34 studying virulence mechanisms, investigating differences between clinical isolates as well as
35 for preliminary investigation of the efficacy of antimicrobial compounds, for a wide range of
36 both Gram-positive and Gram-negative bacteria¹⁻¹², fungi¹³⁻¹⁹ and viruses²⁰⁻²². The use of
37 waxworms as a model host has many advantages. The waxworms themselves are cheap and
38 easy to obtain from commercial insect suppliers, and can be housed in large numbers to allow
39 for greater study sizes at low cost. Waxworms possess an innate immune system that contains
40 many analogous functions to that seen in humans, including phagocytosis and the production

41 of antimicrobial peptides and reactive oxygen and nitrogen species²³. Unlike other non-
42 mammalian model organisms, such as *Caenorhabditis elegans*, *Danio rerio* and *Drosophila*
43 *melanogaster*²⁴⁻²⁷, waxworms can be incubated at 37°C which allows for the study of
44 clinically relevant human pathogens at a temperature that mimics the human host. Finally, as
45 insects, *G. mellonella* are not currently subject to the same ethical restrictions that small
46 mammalian models are, meaning there is a low barrier to entry for researchers wishing to
47 move their studies into a model host.

48 Infection of waxworms is typically carried out on 5th instar insects, when the waxworms are
49 at their largest, typically around 2cm in length and 100mg in weight. The most common
50 method of infection is by injection into the haemocoel through the last proleg of the insect;
51 methods for injection vary between laboratories. One method is to immobilize the needle
52 itself and then place the waxworm onto the needle for injection. Another more favoured
53 method is to immobilise the waxworms between the operator's fingers²⁸ and place the needle
54 into the insect's proleg, lifting the needle away from the operator with the insect attached
55 before pushing the plunger on the syringe. Both of these injection techniques present a hazard
56 to the researcher and can result in needle stick injury and possible infection.

57 A recent article highlighted the use of a stab-proof glove to reduce the chance of this type of
58 injury, while immobilising the waxworms over a pipette tip fixed to some paper²⁹. We have
59 tried this technique, and found that it reduced the efficiency of injection, from 3-4 infections
60 per minute to 1 infection per minute, resulting in a lower injection rate and a more labour-
61 intensive assay. Because of this, we investigated the possibility of using a simple restraining
62 device to hold waxworms in place for injection, in a way that removes the operator's hand
63 from the vicinity of the needle, allowing for maximum mobility and safety of the operator.

64

65 **Materials and methods**

66 *Preparation of bacteria*

67 The *Staphylococcus aureus* isolate XEN36³⁰ (Perkin Elmer) was grown overnight with
68 shaking at 200rpm in Tryptic Soy broth (Oxoid) at 37°C. Cells were washed twice in
69 phosphate buffered saline (PBS) (Sigma-Aldrich) and then resuspended in PBS to an optical
70 density at 600nm (OD₆₀₀) of 1, equivalent to approx. 5×10^9 CFU ml⁻¹. Resuspended cultures
71 were serially diluted and plated onto Tryptic Soy agar (Oxoid) to retrospectively determine
72 the bacterial counts used for injection. Inoculation doses were drawn into 1 ml ultra-fine (29
73 gauge) needle insulin syringes (BD, Wellington) for injection into the waxworms. Groups of
74 waxworms were injected with 20 µl of either approx. 5×10^7 CFU ml⁻¹, 5×10^8 CFU ml⁻¹ or
75 5×10^9 CFU ml⁻¹ *S. aureus* XEN36.

76 *Selection, infection and monitoring of G. mellonella waxworms*

77 5th instar waxworms were selected based on consistency in size and split into eight groups of
78 12. Four groups were injected with either PBS or doses of 10^5 - 10^7 CFU *S. aureus* XEN36
79 using the most common technique of grasping the waxworms between the operator's thumb
80 and index finger and injecting into the waxworm's last proleg. The remaining four groups
81 were injected with either PBS or doses of 10^5 - 10^7 CFU *S. aureus* XEN36 using the newly
82 described restraining device (which we have dubbed the '*Galleria* Grabber'), which
83 comprises a 12 cm x 9 cm kitchen sponge and a large bulldog clip (approx. 50 cm) (Fig. 1A).
84 To comfortably restrain the waxworms, the sponge was folded in half and secured using the
85 bulldog clip (Fig. 1B). The open ends of the folded sponge were peeled back and held in
86 place (Fig. 1C). Next, a waxworm was placed within the sponge and held in place while the
87 open end of the sponge was released (Fig. 1D). Once the waxworm was securely held in
88 place, the insulin syringe was inserted into the haemocoel via the insect's last proleg (Fig.

89 1E). Once the needle was in place the waxworm was released from the restraining device
90 (Fig. 1F). If the needle is correctly placed, the waxworm remains attached to the needle of the
91 syringe. Once the needle had been securely inserted into the waxworm, the insect was
92 removed from the restraining device and the plunger of the syringe pushed down to inject the
93 desired inoculum.

94 Once injected, waxworms were housed in individual wells of 24 well tissue culture dishes
95 (Nunc) with the lids taped down to ensure against escape. These dishes were placed inside a
96 secondary container to ensure containment. Waxworm mortality was monitored over 5 days.

97

98 **Results and discussion**

99 We observed no differences in the infection dynamics between the groups of waxworms
100 injected with *S. aureus* XEN36 after restraint using the novel ‘*Galleria* Grabber’ device
101 described compared to restraint by holding the waxworms between the operator’s thumb and
102 index finger. For both restraint techniques, we observed no mortality from the waxworms
103 injected with PBS (Fig. 2). In contrast, the majority of waxworms injected with approx. 10^7
104 CFU *S. aureus* XEN36 died within 24 hours (Fig. 2). We observed a dose dependent
105 mortality for waxworms injected with *S. aureus* XEN36, with 66% of waxworms injected
106 with approx. 10^6 CFU succumbing to infection (Fig. 2). No mortality was seen after injection
107 with 10^5 CFU *S. aureus* XEN36 (Fig. 2).

108 The ‘*Galleria* Grabber’ allows for easy injection of a large number of waxworms (approx. 3
109 per minute), while greatly reducing the opportunity for the operator to suffer a needle stick
110 injury. With the increasing popularity of waxworms as a model host for studies involving
111 dangerous human pathogens¹², including clinical and/or drug-resistant isolates, protecting

112 researchers from accidental laboratory infection is of great importance. While the use of a
113 stab-resistant glove addresses this issue, it does compromise the speed at which waxworms
114 can be injected. With this new restraint method we were also able to inject smaller waxworms
115 with ease. Most importantly, the new methodology described removes the operator's hand
116 from the vicinity of needles loaded with pathogenic/drug-resistant microbes, allowing for
117 maximum mobility and safety of the operator without compromising the speed of the assay.

118

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121

122 **Disclosure of interest**

123 The authors report no conflicts of interest.

124

125 **References**

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213

214 **Figure legends**

215 **Figure 1. Injection of waxworms using a novel restraint device.**

216 The ‘*Galleria* Grabber’ restraint device is comprised of a 15mm thick sponge and bulldog
217 clip (A). The sponge is folded in half lengthways and secured within a bull dog clip with the
218 open end facing outwards (B). The open ends of the folded sponge are peeled back and held
219 in place (C). The waxworm to be injected is placed within the sponge and held in place while
220 the open end of the sponge is released. The closing of the sponge secures the waxworm in
221 place for injection (E). Once the needle is placed, the syringe is lifted with the waxworm in
222 place and the plunger is pushed to inject the desired inoculum (F).

223 **Figure 2. Survival of waxworms injected with varying concentrations of *S. aureus***

224 Waxworms (n=12 per group) were infected with varying concentrations of *S. aureus* XEN36
225 by injection into the haemocoel via the last proleg while restrained either between the thumb
226 and index finger of the operator (solid lines), or using the ‘*Galleria* Grabber’ restraint device
227 (dashed lines), and survival measured over 5 days.



