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Short Research Communications

Cost-effective bead-based method for high-throughput homogenization of individual small arthropods

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Molecular tools are critical for the analysis of agents of vector-borne diseases in both the hosts and vectors of these agents. A pre-requisite for such analyses is effective methods for the extraction of high quality, inhibition-free nucleic acids. Arthropods are the vectors of multiple infectious disease agents and the extraction of nucleic acids from arthropods requires adequate disruption of the exoskeleton and maceration of tissues to release nucleic acids from the cells. Automation of small arthropod maceration is particularly problematic as these specimens have buoyant bodies and hydrophobic exoskeletons which make them difficult targets for standard beads used in many mechanical tissue disruption methods. Incomplete homogenization results in reduced nucleic acid yields and limits the ability to detect infecting organisms. The use of efficient high-throughput processing techniques for disease vectors is critical for the screening of field collected or laboratory test specimens.

The homogeneous disruption of individual small arthropod vectors of zoonotic diseases such as biting midges (*Diptera*), fleas (*Siphonaptera*), or ticks (*Ixodida*) is typically done manually with pestles in microcentrifuge tubes, or with Dounce tissue grinders[1–3]. Both the methods are labor intensive and can result in: (a) inconsistent sample homogenization; (b) significant volume loss due to adhesion of sample and buffer to the pestle that is then removed from the tube; and (c) introduce the possibility of sample cross contamination as homogenization takes place in open tubes. Other methods capable of homogenizing single small arthropod in high-throughput formats include mechanical agitation with either silica (400 to 800 µm) or zirconium (200 µm) beads[4, 5], solid stainless steel ball bearings[6, 7], tungsten carbide beads (Qiagen)[8], and gold-plated tungsten hollow core beads[9] (Spirit River Inc.). The silica and zirconium beads require multiple beads per well and vigorous agitation. Use of single solid tungsten carbide bead and the gold-plated tungsten bead work well in closed tubes, however, the cost may be prohibitive.

Here we demonstrate the use of 4 mm diameter acid etched gold-plated hollow-core brass beads (Brite Beads™, Spirit River Inc.) for small arthropod homogenization (Fig. 1). These gold-plated brass beads are typically used in making fly fishing lures and can be purchased through fishing/sporting goods suppliers. Both the gold-plated tungsten bead and the gold-plated brass bead have a cylindrical hole through the length of the bead. The hollow core may reduce resistance of the bead traveling through liquid so that minimal force is required to homogenize the samples. This allows the small hydrophobic arthropod to make contact with the bead more efficiently instead of being deflected from and around the sphere during mechanical agitation. In 2007, Kato and Mayer[9] successfully used gold-plated tungsten hollow-core beads for individual *Culicoides* homogenization and now report a more cost-effective solution using gold-plated brass beads for homogenization of midges, ticks, and fleas. The method was validated by performing the homogenization, RNA extraction, and real-time PCR detection with 92 *Culicoides* specimens in a 96-well plate format (4 blank control wells).

The gold-plated brass beads are less dense than the tungsten equivalent and tested because of their significant cost savings. Before use, these beads were cleaned and surface sterilized by washing with 10% detergent (Micro 90, International Products Corp., Burlington, NJ) and sterile MilliQ water (4 times) followed by 5 min in 95% ethanol before air-drying in a clean laminar flow hood. The beads were then handled with surface sterilized forceps (Dumont # 5) and placed one per well in a 96-well plate (1.2 ml Microtube Rack System; Life Science Products, Frederick, CO) followed by 150 µl lysis buffer RLT (Qiagen Ltd., Valencia, CA).

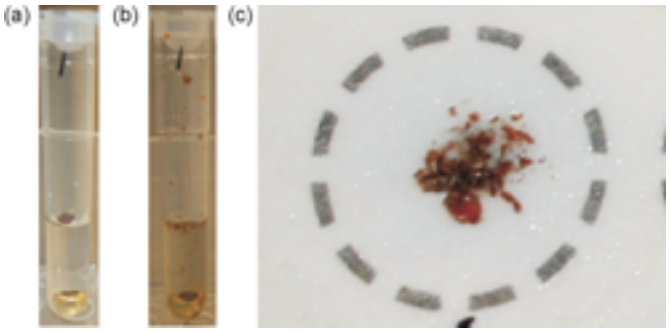

Fig. 1: A single 1.3 ml tube with Brite Bead™, 150 µl RLT lysis buffer and female *Rhipicephalus sanguineus* tick is shown before homogenization in Panel (a); after homogenization in Panel (b); and Panel (c) shows the same remnant of the homogenized tick from panel (b) displayed on filter paper.

Tissue homogenization was performed by adding 150 µl buffer RLT (Qiagen RNeasy 96 kit; with 0.1% β-mercaptoethanol, and 13.3 ng/µl carrier RNA) to each well containing a sterile bead and a single midge. The tubes were sealed with 8-strip caps (Life Science Products) and secured in a Tissue Lyser with 96-well adaptors (Qiagen, Inc.). Mechanical agitation was performed two times for 1 min at 25 rotations/sec. After homogenization, the rack was centrifuged for 2 min at 2000 × g. The remainder of the RNA extraction protocol was performed as described by the manufacturer with all the wash steps performed on the QIAvac 96 Vacuum Manifold (Qiagen, Inc.). The subsequent column drying and elution steps were performed with Qiagen's spin technology protocol with the exception that microtiter plates (Greiner Bio-One Inc., Longwood, FL) were used to support the RNeasy columns during centrifugation in an Allegra centrifuge (Beckman Coulter, Fullerton, CA), at 2000 × g. RNA was eluted in 100 µl RNase free water.

The method for homogenization of single, small arthropods described here uses a single bead per sample in a 96-well format with disruption by mechanical agitation. This allows for homogeneous sample disruption and high volume recovery with ease of bead handling and no cross contamination of samples.

The quality of *Culicoides* RNA was assessed by SYBR green real-time PCR (Quantitect SYBR Green RT-PCR kit, Qiagen, CA) of the *Culicoides sonorensis* actin gene CS-Act1D-F686, TATGCCTTACCACATGCT ATCC and Act1D-R805, AATTTCACGTTCGGC AGTTG[10]. Real-time PCR of the actin gene in *Culicoides* demonstrated that the RNA target remains intact and no PCR inhibiting contaminates are eluted with the RNA. CT values averaged 21.19 (CT range of 20.04–23.17) with a standard deviation of 0.724 (n = 92). This narrow range of CT values shows consistency in arthropod disruption and RNA extraction, with the variation noted likely a reflection of the differences in size and weight of the individual midges.

Unlike midges and fleas, ticks can vary greatly in size and exoskeleton complexity, so, the following variations were made with the protocol to accommodate these differences (Fig. 1). Tick exoskeletons vary in thickness due to size and species so the specification of the mechanical agitation step was varied from a total of 2–5 times for 1 min at 25 rotations/sec. Complete tissue disruption was monitored after every 1 min increment. Larger ticks will increase the amount of fragmented exoskeleton in solution and this may obstruct the flow of the extraction column, therefore, an additional 25–50 µl of lysis buffer was added before homogenization. After homogenization the tubes/racks were centrifuged at 2000 × g to pellet particulate matter and 150 µl of supernatant transferred into a new tube containing 150 µl ethanol (70% as specified by the RNeasy kit).

The use of gold-plated brass beads for arthropod homogenization represents a cost-effective alternative to manual methods or methods employing more costly beads for homogenization. Tissue disruption can be performed in single tubes or in 96-well formats depending on the adaptors and capacity of the mechanical agitation unit. A unit with a two rack capacity can process two 96-well plates (192 samples) in 2 to 5 min, rather than 2 to 5 min per sample (approximately 384 to 960 min) by manual methods. The cost of 192 gold-plated brass beads is approximately one-third to one-tenth the price of hollow core or solid tungsten alternatives, respectively. The method described can be easily adapted for the extraction of either RNA, DNA, or total nucleic acids from small arthropods to facilitate molecular analyses for several vector-borne disease agents, including viruses, bacteria, and protozoans, that infect humans and veterinary species.

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Disclosure statement

No competing financial interests exist. The use of trade names in this document does not constitute an official endorsement or approval of the use of such commer-

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REFERENCES

1. Carpenter S, Wilson A, Barber J, Veronesi E, Mellor P, Venter G, *et al*. Temperature dependence of the extrinsic incubation period of orbiviruses in Culicoides biting midges. *PLoS One* 2011; *6*: e27987.
2. Gamerschlag S, Mehlhorn H, Heukelbach J, Feldmeier H, D'Haese J. Repetitive sequences in the ITS1 region of the ribosomal DNA of *Tunga penetrans* and other flea species (Insecta: Siphonaptera). *Parasitol Res* 2008; *102:* 193–9.
3. Moriarity JR, Loftis AD, Dasch GA. High-throughput molecular testing of ticks using a liquid-handling robot. *J Med Entomol* 2005; *42:* 1063–7.
4. Crowder CD, Rounds MA, Phillipson CA, Picuri JM, Matthews HE, Halverson J, *et al*. Extraction of total nucleic acids from ticks for the detection of bacterial and viral pathogens. *J Med Entomol* 2010; *47:* 89–94.
5. Mayo CE, Mullens BA, Gerry AC, Barker CM, Mertens PP, Maan S, *et al*. The combination of abundance and infection rates of *Culicoides sonorensis* estimates risk of subsequent bluetongue virus infection of sentinel cattle on California dairy farms. *Vet Parasitol* 2012; *187:* 295–301.
6. Kaufmann C, Ziegler D, Schaffner F, Carpenter S, Pflüger V, Mathis A. Evaluation of matrix-assisted laser desorption/ionization time of flight mass spectrometry for characterization of *Culicoides nubeculosus* biting midges. *Med Vet Entomol* 2011; *25:* 32–8.
7. Veronesi E, Mertens PP, Shaw AE, Brownlie J, Mellor PS, Carpenter ST. Quantifying bluetongue virus in adult *Culicoides* biting midges (Diptera: Ceratopogonidae). *J Med Entomol* 2008; *45:* 129–32.
8. Gäumann R, Mühlemann K, Strasser M, Beuret, CM. High-throughput procedure for tick surveys of tick-borne encephalitis virus and its application in a national surveillance study in Switzerland. *Appl Environ Microbiol* 2010; *76:* 4241–9.
9. Kato CY, Mayer RT. An improved, high-throughput method for detection of bluetongue virus RNA in *Culicoides* midges utilizing infrared-dye-labeled primers for reverse transcriptase PCR. *J Virol Meth* 2007; *140:* 140–7.
10. Campbell CL, Vandyke KA, Letchworth GJ, Drolet BS, Hanekamp T, Wilson WC. Midgut and salivary gland transcriptomes of the arbovirus vector *Culicoides sonorensis* (Diptera: Ceratopogonidae). *Insect Mol Biol* 2005; *14:* 121–36.

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