Video Article

Blood Collection for Biochemical Analysis in Adult Zebrafish

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Abstract

The zebrafish has been used as an animal model for studies of several human diseases. It can serve as a powerful preclinical platform for studies of molecular events and therapeutic strategies as well as for evaluating the physiological mechanisms of some pathologies[1].

There are relatively few publications related to adult zebrafish physiology of organs and systems[2], which may lead researchers to infer that the basic techniques needed to allow the exploration of zebrafish systems are lacking[3]. Hematologic biochemical values of zebrafish were first reported in 2003 by Murtha and colleagues[4] who employed a blood collection technique first described by Jagadeeswaran and colleagues in 1999. Briefly, blood was collected via a micropipette tip through a lateral incision, approximately 0.3 cm in length, in the region of the dorsal aorta[5]. Because of the minute dimensions involved, this is a high-precision technique requiring a highly skilled practitioner. The same technique was used by the same group in another publication in that same year[6]. In 2010, Eames and colleagues assessed whole blood glucose levels in zebrafish[7]. They gained access to the blood by performing decapitations with scissors and then inserting a heparinized microcapillary collection tube into the pectoral articulation. They mention difficulties with hemolysis that were solved with an appropriate storage temperature based on the work Kilpatrick et al.[8]. When attempting to use Jagadeeswaran's technique in our laboratory, we found that it was difficult to make the incision in precisely the right place as not to allow a significant amount of blood to be lost before collection could be started.

Recently, Gupta et al.[9] described how to dissect adult zebrafish organs, Kinkle et al.[10] described how to perform intraperitoneal injections, and Pugach et al.[11] described how to perform retro-orbital injections. However, more work is needed to more fully explore basic techniques for research in zebrafish.

The small size of zebrafish presents challenges for researchers using it as an experimental model. Furthermore, given this smallness of scale, it is important that simple techniques are developed to enable researchers to explore the advantages of the zebrafish model.

Video Link

The video component of this article can be found at https://www.jove.com/video/3865/

Protocol

1. Protocol Text

1. Before collecting zebrafish blood, it is necessary to prepare anesthetizing water. Pour ~200 ml of aquarium water into a container with a 500-ml capacity. Add ~200 g of ice chips. The temperature should be about 4 °C. As the ice chips melt, it will be necessary to add more ice chips to maintain a constant temperature near 4 °C.
2. When the anesthetizing water is ready, prepare the materials needed for blood collection. Put a low-retention tip on a P20 pipettor and leave the pipettor where it can be easily accessed. Do not allow the pipette tip to contact any sources of contamination.
3. Cover a Petri dish with a piece of dry gauze. A steel blade and another piece of gauze should be placed in an easily accessible place.
4. A centrifuge adapted for plastic tubes will be needed.
5. When the aforementioned materials are prepared, capture the first zebrafish to be anesthetized with a fishing net and release it into the water that has been prepared for anesthesia. Zebrafish require 3-6 s in chilled water to be anesthetized, depending on the fish. Keep the fish in the cold water until it no longer responds to external stimuli.
6. Using the fishing net, place the anesthetized fish on a prepared piece of gauze, leaving the tail off of the gauze. Fold the gauze over the fish's head and body leaving out only its tail. Put the fish covered with the gauze on the Petri dish.
7. Use the steel blade to make a diagonal incision just between the anal fin and the caudal fin. The blood will start to come out. At this point, it is necessary to work quickly.
8. Gently aspire the blood that comes out with the P20 pipettor (pre-loaded with a low retention tip). The amount of blood that can be collected depends on the size of the fish and to what extent that it was anesthetized correctly. It usually varies from 5 to 20 µl. When the blood stops coming out, gently transfer the aspired blood into a tube.

9. To avoid hemolysis, it is critical that the tube with blood in it be handled very carefully, without any drastic movements until it is placed into the centrifuge.
10. To avoid hemolysis, it is also important that the blood sample be secured in the centrifuge within 10 minutes of blood collection.
11. If necessary, it is possible to combine blood from more than one animal, making a pool. Pooled samples will work fine as long as the delay between blood collection from the first fish and centrifugation does not exceed 10 minutes.
12. When the blood collection is done, centrifuge the blood for 10 minutes at 0.5 g (Eppendorf Centrifuge 5415D).
13. Following centrifugation, the serum is at the top layer of the tube. With a pipette, aspirate the serum, making sure to get just the serum while keeping both layers well divided and stable.
14. Transfer the serum into a new microtube and it is ready to be used in biochemical analyses. The serum can be stored in ice while it wait to start the biochemical analyses.
15. If the serum will not be used immediately, it can be frozen at -18 °C for up to approximately 3 months.

2. Representative Results

It was possible to collect 5 to 20 µl of whole blood from each fish what represents even 4 times more blood than previously described techniques (**Table 2**). Biochemical analysis of total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides were performed after blood collection using this technique. Two groups of both sexes fish were fasted for 24 hours before blood collection to avoid food intake interference. The analyses were done with small-scaled colorimetric tests (Labtest Diagnóstica S.A., Brazil) For total cholesterol and triglyceride analyses, 3 µl of serum were used. For LDL-cholesterol and HDL-cholesterol analysis, 4 µl and 10 µl of serum were used, respectively. These analyses were performed on pooled samples of 10 zebrafish per sample.

Serum lipidic levels were compared between fish that accessed their own eggs and those that, in a bottom covered aquarium, did not have access to their own eggs for an experimental duration of 2 weeks. Serum analysis showed that the serum levels of total cholesterol (with eggs 362 ± 42 mg/dL and without eggs 357 ± 13 mg/dL), HDL-cholesterol (with eggs 91.22 ± 1.79 mg/dL and without eggs 72.14 ± 2.89 mg/dL), and LDL-cholesterol (with eggs 55.68 ± 10.88 mg/dL and without eggs 44.18 ± 9.84 mg/dL) did not differ significantly between the groups. However, triglyceride levels were significantly lower in the experimental group (without eggs 292 ± 64 mg/dL) than in the control group (with eggs 457 ± 25 mg/dL; P = 0.03).

	With access to eggs	**Without access to eggs**
Total Cholesterol (mg/dL)	362.82 ± 73.11	357.69 ± 23.08
LDL – Cholesterol (mg/dL)	55.69 ± 18.84	44.19 ± 17.05
HDL – Cholesterol (mg/dL)	91.23 ± 3.11	72.14 ± 5.01
Triglycerides (mg/dL)	457.64 ± 43.78*	292.36 ± 111.28

Table 1. Cholesterol and triglycerides seric levels for both studied groups (with access to eggs and without access to eggs) expressed in mean ± standard deviation.

*Statistically significant (P=0.03). Student *t* test.

Authors	**Place of incision**	**Harvest method**	**Anesthesia**	**Amount of collected blood**
Jagadeeswaran et. al., 1999 Murtha et al., 2003	Micro dissection posterior to dorsal fin	Micropipette	Not mentioned MS222 3% in cold water	1 a 5 µl 5 a 10 µl
Eames et al., 2010	Decapitation	Micro capillaries tube	MS222 0,02% 28 °C water	5 a 10 µl
Present Study	Incision between anal fin and caudal fin	Micropipette and low retention tips	Water and ice chips	5 a 20 µl

Table 2. Comparison between the previously described blood collection techniques and the one described at the present study.

Discussion

This paper presents a simple technique that allows further blood and serum analysis in zebrafish experiments. This technique has the potential to contribute to future zebrafish hematologic studies requiring blood parameter data. It should also allow for greater applications of the zebrafish as an experimental model.

This technique does not require special skills or implementation of a precise technique. Moreover, it enables up double the amount of blood to be collected relative to other techniques, thereby allowing for the use of fewer fish to obtain the needed amount of biological material. The technique has one critical step, which is that the blood samples be handled carefully as zebrafish blood can incur hemolysis very easily. The time delay between blood collection and centrifugation must be strictly limited. A 10-minute limit should prevent hemolysis. The speed and duration of centrifugation (0,5 *g* for 10 minutes) should also be strictly followed.

Other blood collection techniques were attempted before this technique was developed. However, the number of animals used was large and very small amounts of blood were collected from each fish. This new technique allowed the use of fewer animals, was demonstrated to be feasible with low skill level practitioners, and gave better results than other techniques in terms of the amount of blood collected from each fish.

Disclosures

No conflicts of interest declared.

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