

Procedure for Repeated Ultrasound Guided Liver Biopsies in Non-human Primates

David M. Benedict, Christina Cruzen, Brett Megrath, Brian Knapp, Anthony Celori, Kasey Watson, Kacie Woodward

Altasciences, Seattle, WA, U.S.A

ABSTRACT

Safely collecting any biopsy sample from an internal organ is always a challenge; adding the need to make successive collections increases the difficulty. We chose ultrasound-guided biopsies as the collection method to monitor the changes in the livers of cynomolgus monkeys (*Macaca fascicularis*) over several months. This method offered a minimal level of stress for the animal, a low chance of complications, and a short recovery period.

With the animal under chemical anesthesia using a ketamine/xylazine mixture, the technician used the ultrasound system's 2D mode to visually identify the liver and gallbladder while inserting a 16-gauge biopsy needle. A percutaneous biopsy specimen was then collected from the right side of the liver. The procedure consistently yielded a 1.5cm length of useable liver tissue for analysis.

Following the procedure, only minimal pain management was needed, with 0.1 mg/kg of meloxicam being administered for three consecutive days. After performing 342 ultrasound-guided biopsies across 189 animals, with some animals having received four biopsies over the course of 70 days, we did not have a single major issue during the biopsy or recovery.

Ultrasound-guided liver biopsies have proven to be a minimally invasive, well-tolerated, and a safe method for repeated biopsy collection from the liver in non-human primates.

EQUIPMENT AND MATERIALS

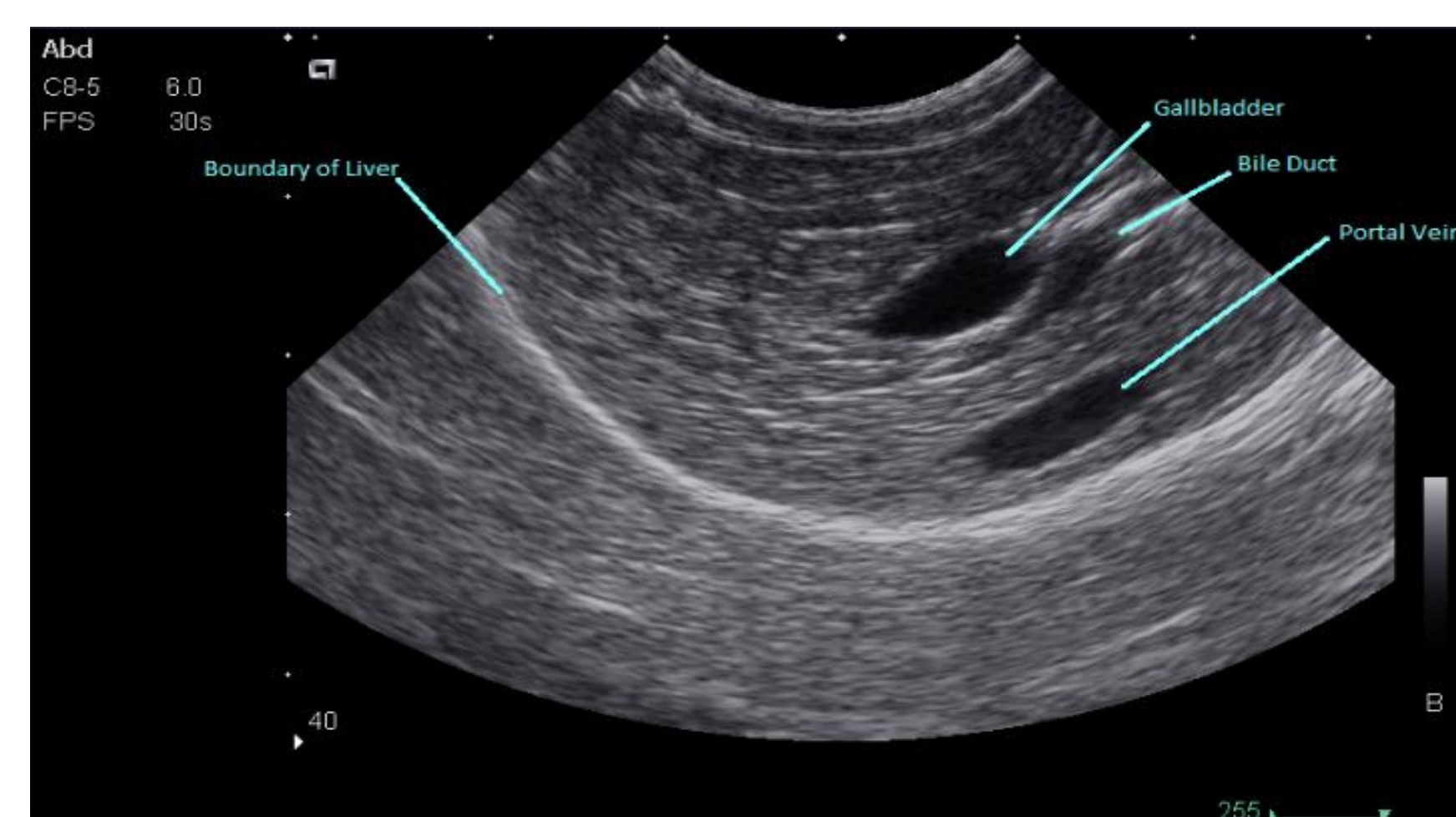
- Surgical scrub (i.e. chlorhexidine scrub)
- Gauze sponges
- 70% isopropyl alcohol
- Antiseptic solution (i.e. diluted chlorhexidine solution)
- Clippers with a #40 clipper blade
- Scrubs
- Bouffant cap
- Disposable face shield
- Disposable surgical mask
- Surgical blade #11
- 16 gauge or smaller SuperCore or Tru-Cut biopsy needle, or equivalent
- Ultrasound with appropriate probe
- Sterile ultrasound gel or sterile lubricant
- Tissue glue
- Tuberculin syringes
- Ketamine/xylazine (95.2/4.8 mg/ml)
- Antisedan (5mg/ml)
- Meloxicam (5mg/ml)
- Sterile gloves
- Sterile drape

PROCEDURE

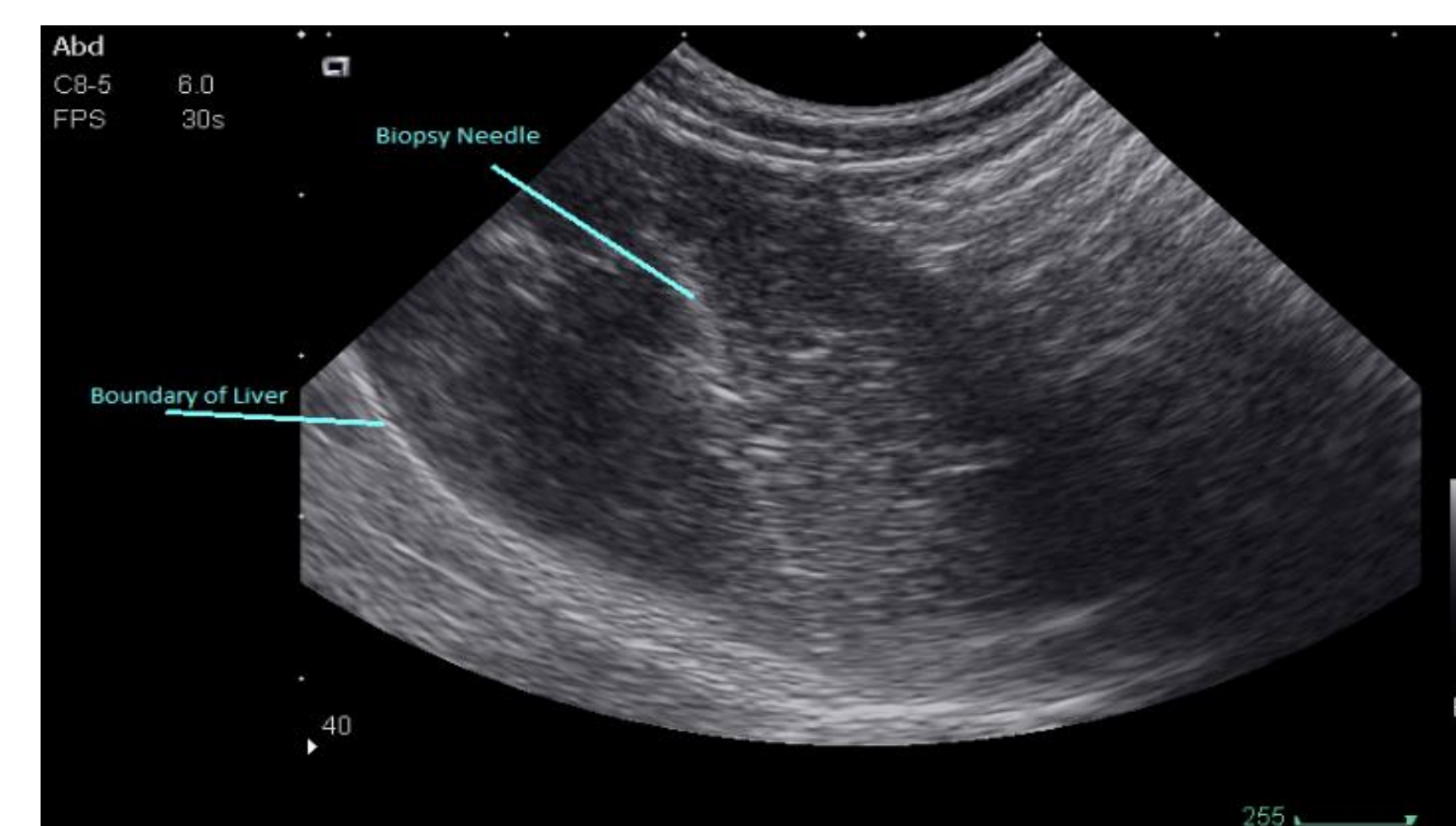
1. Anesthetize the animal with 13 mg/kg of ketamine/xylazine.
2. Administer pre-analgesics 0.1 mg/kg of meloxicam.
3. Place the animal in a dorsal recumbency and surgically prepare the cranial abdomen by first shaving the area; then aseptically prep with surgical scrub and isopropyl alcohol.
4. Examine the upper right quadrant of the abdomen with the ultrasound. Locate the liver and the major structures associated with it (i.e. gall bladder, portal vein, vena cava and aorta). Locate a portion of the liver that is away from these structures for sampling.
5. Place sterile fenestrated drape over the collection site.
6. Using a scalpel blade, make an incision through the skin in a location that provides access to an area of the liver away from vital structures. Typically, this is approximately 1-2 cm caudal to the xiphoid process, and to the right of midline.
7. Arm the biopsy needle, insert it into the skin opening and advance it slowly through the subcutaneous and abdominal wall layers into the liver with ultrasound guidance.
8. Collect a core sample of liver tissue. Remove the needle and place the core sample in the collecting receptacle.
9. Apply pressure to the skin incision and close using tissue glue. Monitor the biopsy site and assess the abdominal cavity with the ultrasound for signs of hemorrhage after the skin is closed. (Signs of marked bleeding will be observable by ultrasound after a few seconds of the procedure.)
10. Administer antisedan at 0.25 mg/kg to reverse the xylazine and help speed recovery from sedation.
11. Administer meloxicam perioperative at 0.1 mg/kg an analgesic for two days.
12. Observe biopsy site and animal demeanor for approximately 5-7 days following the procedure.

IMAGE OF THE LIVER

Using 2D ultrasound, the liver, gall bladder, and large vasculature structures can easily be visualized. The fluid in the gall bladder, veins, and arteries make them show up as a black mass, and the boundaries of the liver appear as a more solid white line.



With the ultrasound, it is possible to continually monitor the progress of the needle, which is seen as a white line, and its position relative to other structures.

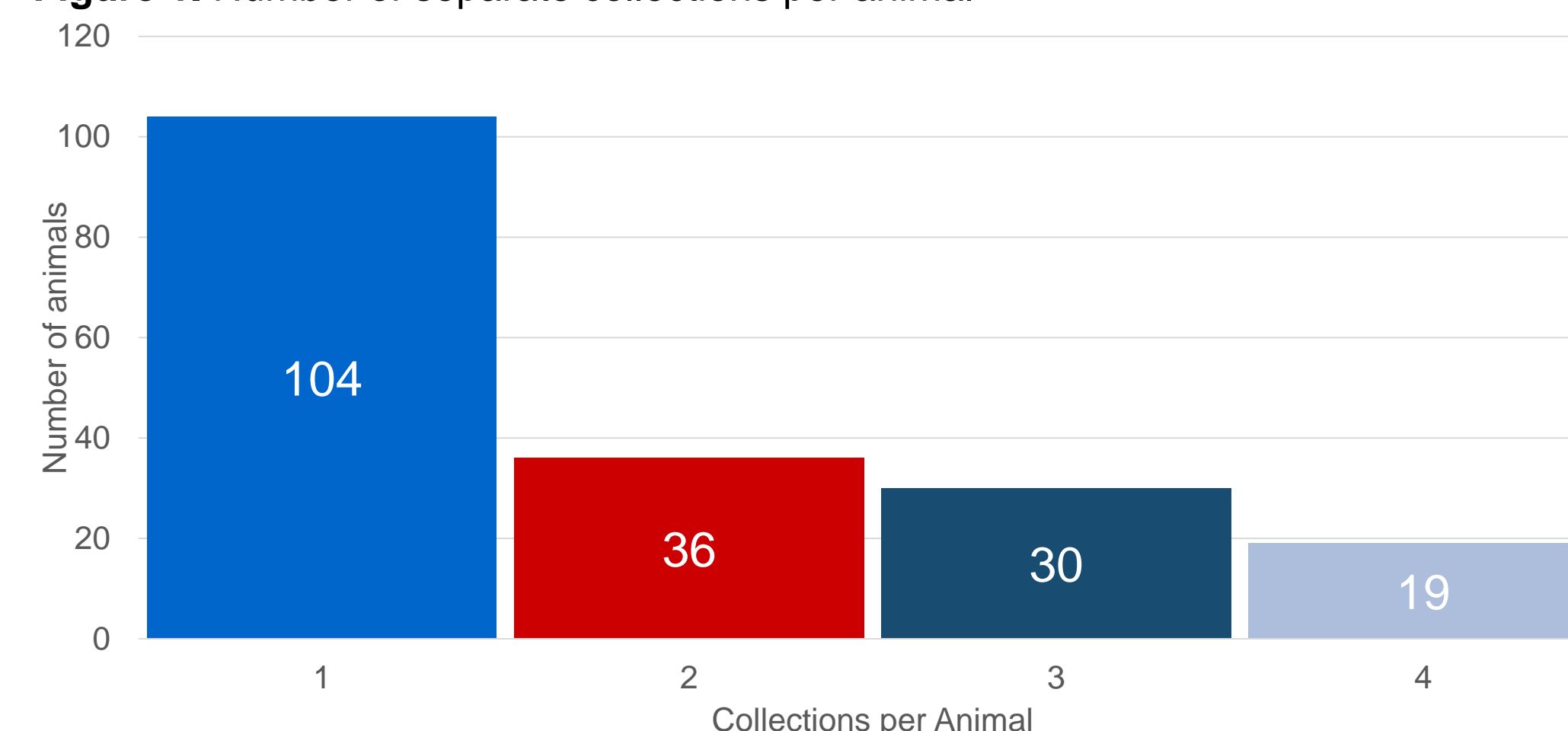


RESULTS

Over the course of two years we needed to collect biopsy samples from the livers of cynomolgus monkeys to monitor change. The amount of tissue needed was relatively small, only about 1.651 mm (16 gauge) by 1.5 cm, so it was decided that the safest and most efficient method of performing the collections was with the use of 2D ultrasound and a biopsy needle.

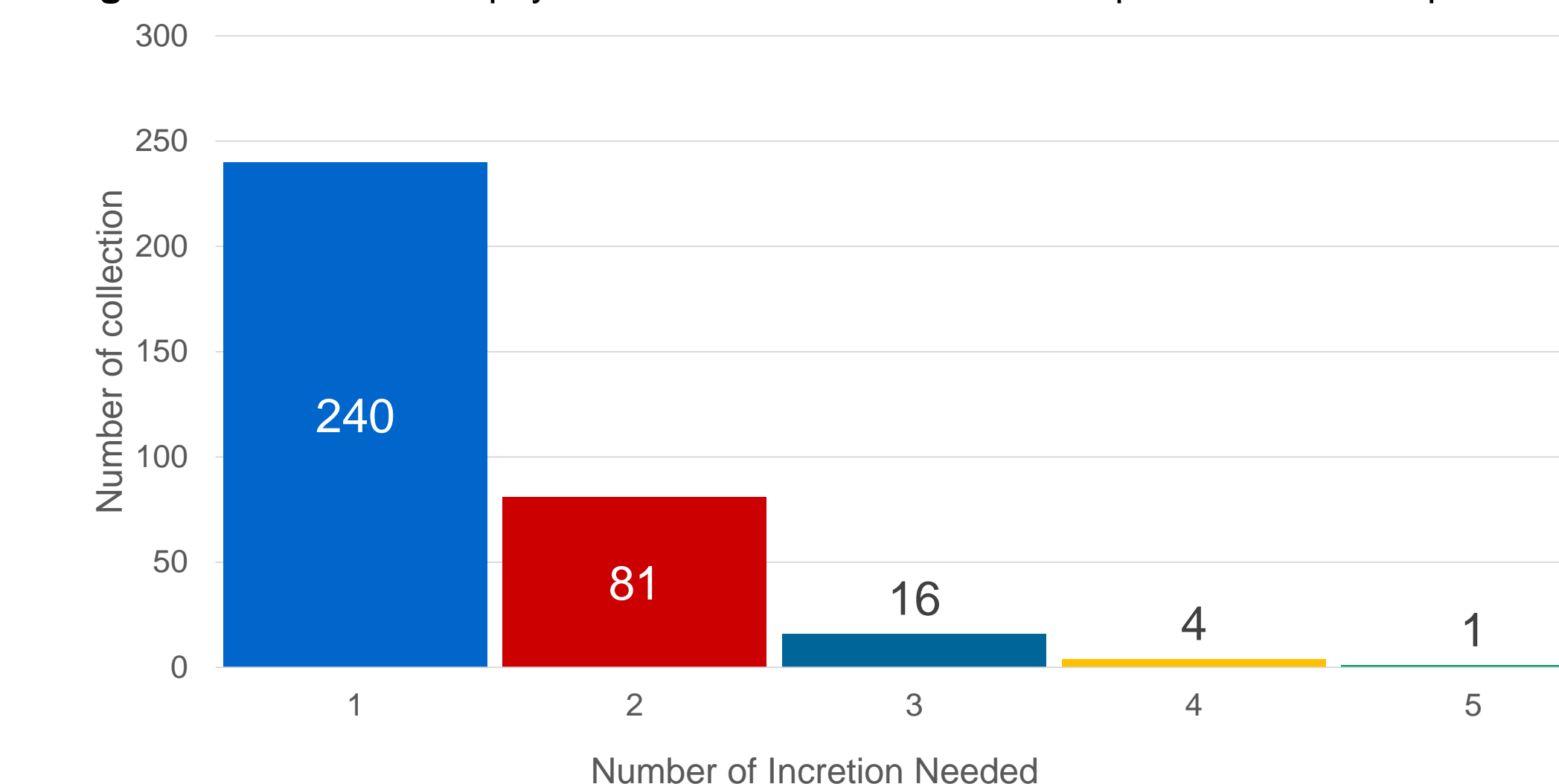
Collections were made from 189 prescreened animals. Several factors determined how frequently a biopsy sample needed to be collected, with the vast majority only needing one collection; some, however, required up to four collections over the span of 70 days.

Figure 1. Number of separate collections per animal



With the multiple collections, 342 biopsy samples were collected across all animals. With the tissue requirements for the assays it was essential, provided the welfare of the animal was not jeopardized, to meet a minimum length requirement of 1.5 cm. When the initial collection did not yield enough liver tissue, additional insertions of the needle were performed. 70% of collections only required one attempt and in 93% of the collections, the minimum length requirement was achieved with two or fewer insertions.

Figure 2. Number of biopsy needle insertions needed to acquire a 1.5cm sample



The collection procedure was well-tolerated by the animals, but a few minor complications did occur. After 11 collections extra support was provided to animals that experienced extended recovery from sedation. This consisted of one or a combination of the following treatments: an additional dose of antisedan, subcutaneous saline, or dextrose administered via IV.

Due to the relative positions of some anatomical structures around the liver, a second incision was rarely required to access it from a different angle. Also, penetrating the hepatic capsule proved difficult on some animals.

Minor complication encountered	
Animals that needed extra support during recovery	11
Second incision need for collection	4
Difficulty penetrating hepatic capsule	3

CONCLUSIONS

With this procedure, we are confident we have a safe method for repeated biopsy collections from the liver in non-human primates. Using only 2D ultrasound, it is possible to identify all major structures in and around the liver, allowing for the safe insertion of a biopsy needle. The procedure can be done quickly and since it is minimally invasive, it allows for the collection to be completed with only chemical anesthesia.

With only a few minor complications that were simple to treat, and no ill effects from successive collections, ultrasound-guided liver biopsies are well-tolerated and safe method for collecting samples from the liver.