



# OPEN Versatile application of fast green FCF as a visible cholangiogram in adult mice to medium-sized mammals

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An aqueous solution of a common food dye, Fast Green FCF (FG), mimics cholyl-lysyl-fluorescein to visualize embryonic bile flow via single peritoneal injection into intrauterine mouse embryos. Despite its efficacy in embryos, its suitability for adult mice and small to medium-sized mammals remained uncertain. In this study, we investigated FG cholangiography in adult mice, dogs, and goats. The results demonstrate that FG injection enables progressive cholangiography in these species, highlighting its versatility across different animal models without necessitating specialized equipment. To further evaluate diagnostic utility, FG cholangiography was performed in various mouse models of bile flow disorders. FG successfully visualized dilated lumina in the extrahepatic bile duct of BDL mice and revealed aberrant luminal structures in the gallbladder walls of *Sox17<sup>+/-</sup>* or *Shh-cre*; *Sox17<sup>fllox/-</sup>* mice. In *Mab21l1<sup>-/-</sup>* mice with contracted gallbladders, FG influx was limited to the gallbladder neck. Moreover, stereomicroscopic video analysis of FG influx into the gallbladder post-fasting revealed differences in gallbladder wall state and its bile composition between *Sox17<sup>+/-</sup>* and wild-type mice, suggesting the potential for detecting variations in gallbladder stored bile properties. These findings underscore the efficacy of FG in facilitating progressive cholangiography across mammalian species.

**Keywords** Cholangiography, Bile flow, Gallbladder, Mouse, Dog, Goat

## Abbreviations

BDL	Common bile duct ligated
FG	Fast green FCF
ICG	Indocyanine green
NIR-II	Second near-infrared region
PBG	Peribiliary gland

Bile is produced in hepatocytes and then transported through the intrahepatic bile ducts to the hepatic and common hepatic ducts for extrahepatic transport. In animals with a gallbladder, bile is transported through the cystic duct to the gallbladder for storage<sup>1,2</sup>. During the inter-meal period, the bile in the gallbladder is concentrated through its walls, and the concentrated bile is released into the duodenum via the common bile duct by meal stimulation. If this bile flow from the liver to the duodenum is disrupted, biliary atresia occurs, leading to severe hepatic dysfunction and significant morbidity and mortality<sup>3</sup>.

For the pre- and post-operative evaluation of bile flow in biliary diseases, contrast agents are widely used in medical imaging to enhance the visibility of internal structures or fluids during diagnostic procedures<sup>4,5</sup>. In humans, gadolinium-based agents are commonly used for MRI, while iodine-based agents are used for X-ray and CT scans for perioperative evaluation of the biliary tree. Since progressive cholangiography is very

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