# Evaluation of a Minimally Invasive Method for the Collection of Bile in a Phase I Human Mass Balance Study of Normal Healthy Subjects

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## Abstract

**Purpose:** To fully characterize the metabolism of a chemical entity/investigational drug in humans, hepatobiliary elimination pathways may need to be investigated. Traditional bile collection methods in Phase I trials have required nasoduodenal intubation which can be technically challenging. Recent innovations have provided a minimally invasive method for bile collection in humans which in turn can be utilized to characterize bile-containing parent drug and metabolites. We conducted a human mass balance (HMB) study in normal healthy volunteers (NHVs) in which a non-invasive method was utilized to confirm biliary excretion of a metabolite not detectable in feces. **Methods:** A previously published method using the Entero-Test® capsule/string device (HDC Corporation, Milpitas, CA) was adapted for use in this HMB study. The procedure was initially tested with 12 consented volunteers, for verification and training purposes. The method was then used to collect bile from 8 NHV in the HMB study. During the study, immediately prior to receiving an IV $^{14}$C-drug dose, 8 subjects swallowed the Entero-Test® capsule/string device. The device consists of a tethered, absorbent string inside a weighted capsule. One end of the string tether was taped to the subject's cheek, and the subject swallowed the capsule. The string was kept in place for up to 6 hours post-dose in the duodenum, thereby acting as a wick to collect bile. At the end of the sample collection interval, the string was retrieved and bile components extracted for analysis. The resultant extracts were counted by liquid scintillation counting (LSC) to determine the amount of radioactivity present. Extracts were further analyzed using liquid chromatography/mass spectrometry (LC/MS) to determine the $^{14}$C-drug metabolite profile. **Results:** All 8 subjects tolerated the procedure well with only minor discomforts, such as sore throat, reported. The procedure enabled identification and confirmation of metabolites of interest in human bile, including several metabolites not detected in feces. **Conclusion:** The Entero-Test® capsule/string method was shown to be a minimally invasive and well tolerated procedure which allowed collection of bile and confirmation of biliary metabolite excretion.

## Purpose

To fully characterize the metabolism of a chemical entity/investigational drug in humans, hepatobiliary elimination pathways may need to be investigated. Traditional bile collection methods in Phase I trials have required nasoduodenal intubation which can be technically challenging. Recent innovations have provided a minimally invasive method for bile collection in humans which in turn can be utilized to characterize bile-containing parent drug and metabolites. We conducted a human mass balance (HMB) study in normal healthy volunteers (NHVs) in which a non-invasive method was utilized to confirm biliary excretion of a metabolite not detectable in feces.

## Methods

The Entero-Test® capsule/string device (HDC Corporation, Milpitas, CA; Figure 1) was originally developed as a test for intestinal parasites and other pathogens. The device was repurposed for the collection of bile as outlined in previously published journal articles.[1,2] This technique for bile collection was also utilized in this HMB study.

The device consists of a tethered, absorbent string inside a weighted capsule. One end of the string tether was taped to the subject's cheek, and the subject swallowed the capsule with at least 240 mL of water. Once swallowed, the subjects were asked to jump up and down in place 5 to 10 times in order to help the capsule travel into the stomach.

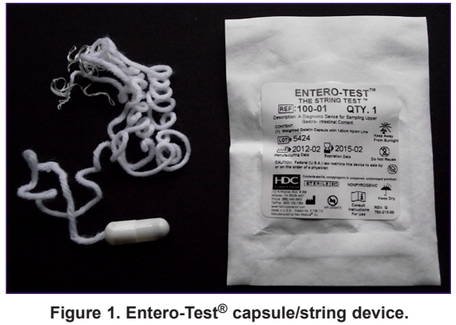


**Figure 1. Entero-Test® capsule/string device.**

The sample collection procedure was initially tested at the Covance Clinical Research Unit with 12 consented volunteers, for verification and training purposes. The method was then used to collect bile from 8 NHV in the HMB study from 0-6 and 6-12 hours postdose. The sample collection intervals scheduled were determined based on metabolite profiling and ID data obtained from animal ADME studies conducted with the investigational product.

Immediately prior to receiving an IV $^{14}$C-drug dose, 8 subjects swallowed an Entero-Test® capsule/string device. During each of the collection intervals, subjects were allowed to drink water ad libitum and were provided meals that had been specifically selected to avoid any foods that may stain or stick to the string. In previous published methods, concomitant medications were administered to stimulate the contraction of the gallbladder. As subjects were allowed to eat and the investigational product was a known gastric stimulator, the administration of such medications was deemed not necessary.

The string eventually travelled into the duodenum during each collection interval, thereby acting as a wick to collect bile. Subjects were instructed that if they experienced any discomfort or difficulty eating with the string, it could be removed, however all subjects kept the string in place for the entire time for each 6-hour interval.

At the end of each sample collection interval, the string was retrieved by tilting the subject's head backward and gently pulling the line upward and out. Following a period of approximately 3 hours following ingestion, the capsule shell is designed to disintegrate and the small stainless steel ball used to weigh the capsule is then eliminated in the feces. Subjects were allowed a 15-minute rest period between the extraction of the 0-6 hour string and the insertion of the 6-12 hour string. Immediately after collection, the line was placed in an appropriately labelled cryovial and a visual inspection was performed of the string to determine if there was sample present.

Bile components were extracted for analysis by the Covance Drug Metabolism and Pharmacokinetics Department. The resultant extracts were counted by liquid scintillation counting (LSC) to determine the amount of radioactivity present. Extracts were further analyzed using liquid chromatography-mass spectrometry (LC-MS) to determine the $^{14}$C-drug metabolite profile. Results of the sample analysis are presented in Posters #T3353 and #T3354.

## Results

All 8 subjects tolerated the procedure well with only minor discomforts, such as sore throat, reported. All subjects were able to eat and drink with the strings in place and the strings did not appear to affect any of the activities of daily living.

Visible staining of the bile string, which was likely attributed to the presence of bile, was observed in all 8 strings during the 0-6 hour collection interval and in 5 of the 8 strings during the 6-12 hour collection interval. Staining of the string occurred in varying degrees during each collection interval. A degree of staining observed on each string is noted in Table 1.

**Table 1. Color Staining of the Bile Strings**

| Subject ID | 0-6hr | 6-12hr |
|---|---|---|
| 12001 | + | ++ |
| 12002 | ± | ++ |
| 12003 | +++ | ++ |
| 12004 | +++ | ± |
| 12005 | + | + |
| 12006 | +++ | ++ |
| 12007 | ++ | - |
| 12008 | ± | - |

- no color ± least + partially +++ most

Representative photographs of the retrieved strings during each collection interval that had the least amount of visible staining (Figures 2 and 4) and those that had the most amount of visible staining (Figures 3 and 5) are presented below.

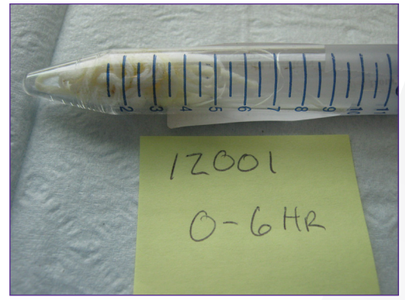


**Figure 2. Subject 12001, 0-6 hr.**

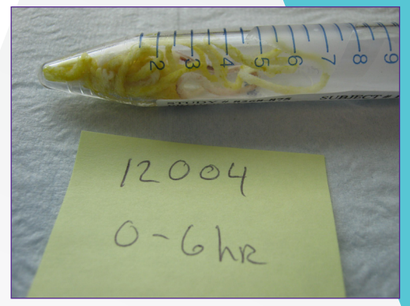


**Figure 3. Subject 12004, 0-6 hr.**

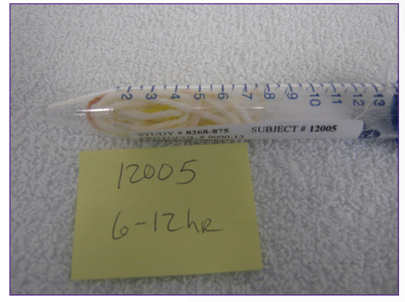


**Figure 4. Subject 12005, 6-12 hr.**


**Figure 5. Subject 12006, 6-12 hr**

The collection and extraction of the bile from the strings enabled identification and confirmation of the metabolites of interest in human bile, including several metabolites not detected in feces.

## Conclusion

The Entero-Test® capsule/string method was shown to be a minimally invasive and well tolerated procedure that allowed collection of bile and confirmation of biliary metabolite excretion. Although this method does not allow for the determination of the percent of radioactivity being excreted via biliary pathways, it is a useful tool to identify any metabolites that may be present and not quantifiable via fecal excretion.

## References

1. Giulia Ghibellini, et. al., Methods to Evaluate Biliary Excretion of Drugs in Humans: an Updated Review, *Mol Pharm*., 198–211 (2006).
2. William J. Guiney, et. al., Use of Entero-Test, a simple approach for non-invasive clinical evaluation of the biliary disposition of drugs, Br. J. Clin. Pharmacol., **72**, 133–142 (2011).

*Presented at AAPS 2014*

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