Appendix 1:

Standard operating protocol:
Measurement of Gastrografin in surgical drain fluid and stool by Dual Energy CT (DECT)

Background
The benefit of DECT is achieved via its ability to distinguish between materials based on their spectral properties; a material's capacity to attenuate x-ray photons at different photon energies. Materials interact with x-ray photons in several ways including the Compton effect and the photoelectric effect[1]. DECT operates on the principle that two different x-ray spectra will cause a material to absorb x-ray photons differently according to these interactions. This particularly applies for the photoelectric effect which is dependent upon a material's properties, such as the atomic number, electron density and K-edge electron energy. The widely used CT contrast agent iodine has a high atomic number ($Z = 53$) which results in a strong photoelectric effect. This photoelectric effect and spectral behaviour can be measured by DECT and can be used to detect and quantify iodine containing substances, such as Gastrografin[2].

DECT scan protocol and sample measurement
Daily 10 mL samples of drain fluid were collected into 50 mL sterile jars and stored at room temperature. All samples were scanned via the same dual source CT system (Somatom Force, Siemens Medical Solutions, Forchheim, Germany) in the same radiology department by the same senior CT radiographer. The samples were placed flat onto the CT table and evenly spaced by 10 cm. The samples were scanned within 4 weeks of sample collection.

The dual energy protocol with acquisition parameters of 80 kV and Qref mAs of 100 mAs on the A tube and sn150 kV and QRef mAs of 67 mAs on the B tube were used to scan each sample. To deliver a constant radiation dose for all scans, no dose modulation was utilised. Each detector was collimated to 128×0.6 mm with a flying focal spot, and a pitch of 0.7 was applied. Images were reconstructed with a dedicated Br40 reconstruction algorithm. Slice thickness and increment were 1.0 mm and 0.7 mm, respectively. Two individual stacks of images for each detector (80 kV and sn150 kV images) and a DE mixed images were reconstructed. The latter will contain weighted information from both detectors with a weighting factor of 0.6 (60% from the 80 kV scan and 40% from the sn150 kV scan) thus approximating regular 120 kV images.

After reconstruction, images were transferred to the same workstation with dedicated commercial post-processing software (Syngo Dual Energy, Siemens Medical Solutions, Forchheim, Germany). The dual energy datasets were analysed using the LiverVNC software where iodine density and HU were measured for each sample without any manual adjustments to the algorithm.

Two separate region of interest (ROI) measurements were used to measure the HU and iodine density (mg/mL) of each sample. The ROI measurements were obtained via coronal reformats to the right and left of the solution midline, each ROI measuring 10 mm^2. All measurements were performed by the same radiologist who remained blinded to the samples.

Samples were re-scanned twice during the course of the study, and if significant discrepancies between results were present, the solutions would be rescanned twice more. The two individual HU and iodine concentration results for each sample was recorded as per the data collection described in the protocol.

Preliminary studies of iodine measurement by DECT
Our preliminary *in vitro* and *ex vivo* studies have contributed to the development of DECT protocol (Appendix 1) to detect the presence of GG in a sample solution of sterile water or surgical drain fluid. The lower limit of detection of a solution containing GG was at a concentration of 0.097%, which correlated to an iodine density of > 1 mg/mL. The upper limit of the true negative levels of GG measured in drain fluid by DECT was derived by calculating the mean (+3 SD) iodine density (mg/mL) in GG negative samples. We have established measurements of iodine density above 1.2 mg/mL to represent a positive result for the presence of GG in a solution measured by DECT. The methodology for the quantitation of GG in biological fluids has been reported in a submitted technical paper looking at serial dilutions and stability of GG *in vitro*.

[1]Coursey CA, Nelson RC, Boll DT, Paulson EK, Ho LM, Neville AM, et al. Dual-energy multidetector CT: how does it work, what can it tell us, and when can we use it in abdominopelvic imaging? Radiographics 2010;30:1037-55.
[2]Johnson TR. Dual-energy CT: general principles. AJR Am J Roentgenol 2012;199(5 Suppl):S3-8.