SECTION: Clinical **Procedure No.** 01243/v1/08/2018
PROCEDURE TITLE: Intestinal permeability test

Review Officer: Senior Scientist

Review Summary: v1

Applicable To: Gastroenterology Laboratory Staff

Date Introduced: 08/2018

Next Review Date: 08/2021

Authority: Director, Gastroenterology and Hepatology

Replaces: New procedure

Key Words: lactulose, rhamnose, intestinal permeability

PURPOSE
The Intestinal Permeability Assessment gastrointestinal test directly measures the ability of non-metabolised sugar molecules to permeate the intestinal mucosa.

OUTCOME
The outcome of the procedure is that an accurate and reliable test is performed.

AUTHORISED TO UNDERTAKE THE PROCEDURE
All Gastroenterology Scientific Laboratory Staff trained in the procedure.

CONTRAINDICATIONS
Relative: Lactulose intolerance.

RISKS AND PRECAUTIONS
Needle stick injury – personal protective equipment is to be worn.

STEPS OF THE PROCEDURE
Background
The patient drinks a premeasured amount of **lactulose** and **rhamnose**. The ratio of plasma lactulose and *l*-rhamnose (L/R ratio) is a measure of intestinal permeability. Lactulose is a large molecule and normally very little is absorbed from the gut. Following a variety of insults the gut becomes more permeable, and lactulose may be absorbed into the blood. The degree of intestinal permeability is reflected in the ratio of the two sugars recovered in a plasma sample collected after 90 minutes.

Materials
- 5 g lactulose MW 342.29 (Actilax, each 5 mL containing 3.3 g lactulose)
- 1 g *l*-rhamnose MW 182.17 (Sigma Aldrich, Food Grade)
- 1 disposable drinking cup
- HPLC-mass spectroscopy

Instructions:
- The patient fasts overnight (10 hours)
- The patient drinks 100 mL solution containing 5.0 g lactulose and 1.0 gram *l*-rhamnose
- 18 mL of peripheral blood is collected into heparinised tubes after 90 mins. Blood will be centrifuged at 2000g, 10 mins, 4 °C.
- Plasma is aliquoted and stored at -80 °C until tested.

Analysis of results:
- Plasma or serum samples are thawed and diluted 1:3 in acetonitrile (9:1 ultrafiltered water) containing 7 µg/mL ibuprofen as an internal standard. Samples are vortexed, sonicated (5 min) and centrifuged (13.3K rpm/17K G, 5 min). Supernatants are transferred to a clean HPLC vial for LCMSMS.
- Standard curves were generated by spiking clean acetonitrile and serum/plasma with rhamnose and lactulose (50K µg/mL – 50 µg/mL), also containing the corrected ibuprofen concentration as an internal standard.
- Samples are run in negative ion mode on an ABSCIEX QTRAP 4000 mass spectrometer coupled to a Shimadzu Nexera X2 autosampler/chromatographer unit. LC is performed using a Zorbax Polaris 5 amino column (150 x 2.0 mm) and a HILIC (‘reverse-reverse’) phase method (Buffer A: Aqueous 0.1% formic acid 10 mM ammonium formate, Buffer B: Acetonitrile 0.1% formic acid). Runs were 15 min.

- LCMS-MS methods were developed and optimised based on fragment mass detection in negative ion mode for the ammonium conjugate of the parent molecule, and are as follows (Q1/Q3; ret time): Rhamnose 209.2/162.2Da and 209/103.1Da; RT 3.95 min. Lactulose 387.0/341.0Da and 387.0/161.0; RT 7.07 min.
- Ibuprofen (internal standard) 251.0/161.0; RT 2.52 min.
- L/R ratio = (percentage L from initial dose/percentage R from initial dose)

EVALUATION METHOD

Ongoing evaluation of any changes to this clinical practice will be coordinated the Senior Scientist and Research Officer.