

Standard Operating Procedure: Collecting Colonic Biopsy Samples for Histopathological Protein, Gene Expression and Epigenetic Analysis

Introduction

Irritable Bowel Syndrome (IBS) is one of the most common gastrointestinal diseases that negatively impacts on sufferer's quality of life. The generation of suitable therapeutics is counteracted by the fact that the etiology of this disease is largely unknown. A recent hypothesis claims activation of the innate and adaptive mucosal immune system reflecting a previous insult or a state of low-grade inflammation. These inflammatory events are discussed as triggers for a neuronal re-modeling, which manifests in clinically evident visceral hypersensitivity, intestinal motility disorders as well as changes in intestinal permeability. The histological examination of endoscopically obtained colonic biopsies could strengthen this hypothesis, as biopsies can be used to monitor inflammatory events in the tissue as well as changes in neuronal plasticity, neurotransmitter alterations and intestinal permeability.

To date no systematic studies have been performed comparing gene expression changes in different locations of the gut of IBS patients compared to healthy control individuals and only few epigenetic studies assessed microRNAs profiles, tiny molecules which fine tune gene expression of protein encoding genes. In addition, epigenetic modification of DNA has not been addressed in IBS which may impact expression by switching genes on or off in a long-term manner.

A total of *four* biopsies should be taken from each site:

1. *Two* biopsies from each site (rectum and descending colon) will be collected in order to enable two of these will undergo histopathological follow up of marker proteins such as enterochromafin markers (TPH1, Serotonin), mast cells (tryptase), T-lymphocytes (CD3,4,8), neuronal cells (HuCD) nerve fibres (PGP9.5) in addition to validation of differentially expressed genes and miRNAs as well as epigenetically modified imprinted genes.
2. The remaining *two* biopsies (i.e. one from each site) will be used for genetic/epigenetic studies by performing differential expression analyses and epigenetic studies.

Depending on local protocols, ideally *x4 biopsies* samples will also be taken from the transverse colon, caecum and terminal ileum, with one from each area used for histological analysis and one used for genetic/epigenetic studies.

Biopsy material

Type of biopsies

- For standard lower colonic endoscopic procedures, following standard, either per oral or per rectal cathartic bowel preparation, as per local guidelines.
- Only mucosal biopsies with standard forceps.

Location of biopsies

- Colon: technically easiest to perform and most consistent data from literature with regard to IBS
- Location: rectum and descending colon.
- *Optional: if possible, depending on local availability, 4 further biopsies can also be taken from the terminal ileum and ascending colon.*

Number of biopsies

- At least *x8 biopsies* from – 4 samples from each of the rectum and proximal descending colon.

Preservation of biopsies

- Formalin-fixation (10%) for immunocytochemistry (2 from rectum , 2 from proximal descending colon).
- Allprotect (vide infra) – (2 from rectum and 2 from proximal descending colon).

Allprotect protocol

Allprotect (Qiagen) Tissue Reagent provides immediate stabilization of DNA, RNA, and protein in tissue samples at room temperature. This preserves the *in vivo* profile of DNA, RNA, and proteins, allowing reliable downstream analysis. Stabilized tissues can be transported at 15–25°C for up to 7 days, or stored at 2–8°C for up to 12 months. For longer storage, stabilized tissues can be archived at –20°C or –80°C.

Preparation for the biopsy samples for Allprotect

2ml tubes (Sarstedt # 72.694.007) filled with 150 µl of Allprotect.

Processing of the biopsy

After taking the biopsies they should be fully submerged in the tube, **ensuring the biopsies are completely immersed in the solution** to avoid them getting stuck to the tube and drying out.

If the samples are to be shipped to another centre, before shipment of biopsy samples one biopsy from each tube is transferred to a new 2ml microtube with inner vial (0.5ml) (Sarstedt, #72.730.007) (labeled the same) filled with 500µl of Allprotect suitable for shipment.

If samples are to be processed locally then they can be stored at 2–8°C for up to 12 months. Longer term storage can be achieved by storing the samples at –20°C or –80°C.

Biopsy summary flowchart

