Laboratory and pathological diagnostic assessment of each Gastroenterology Research Center

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ABSTRACT

New techniques of gastric, small bowel and colonoscopic biopsy followed and added to the abundance of tissue available to pathologists for diagnosis and study of the pathogenesis of GI disease. Today gastrointestinal pathology is accepted as one of the largest sub-specialties within general histopathology and there has been a steady movement away from old style morbid anatomy and histology during the past 50 years to a greater appreciation of cellular pathology. Although new techniques, in particular immunocytochemistry and molecular analysis, have become popular, it is remarkable how the old technique of H&E staining remains the standard for diagnostic purposes, especially in research projects. In this short review we will introduce the laboratory and pathological assessments available at each Gastroenterology and Liver Diseases Research Center.

Keywords: Gastroenterology, Molecular typing, Pathology

Introduction

The primary aim of examining pathologic specimens is to provide physicians and surgeons with essential diagnostic and prognostic information, however, this is not the only purpose of pathological examination, and these specimens serve as important sources of materials for investigating disease processes and also for teaching students (1). In each gastroenterology research center the biopsies and other bodies' fluids samples from every parts of the digestive system, from mouth to colon, in order to diagnose and score the grade and stage of various gastrointestinal (GI) diseases will be collected (Figure 1). In this short review we will discussed the laboratory and pathological assessments for each Gastroenterology and Liver Diseases Research Center.

I. Histochemical sectioning and staining

Most GI mucosal biopsies are ting samples of a large surface area of suspected disease (2). Section paraffin blocks of a small mucosal biopsies (Figure 2), are stained routinely with hematoxylin and eosin (H&E). Additionally, for gastric biopsies, stain for helicobacter-like organisms with Gimsa are also applied.

Meanwhile, trichrome and reticulin staining will use routinely for liver biopsies.
Other techniques revealed to be helpful for diagnostic approaches are include: PAS (periodic acid-Schiff), Amyloid staining (Congo-Red), Argentaffin and argyrophilic stains, PTAH, stains for hemosiderin (Pearls), melanin (Fontanamasson) and calcium (Von Kossa), mucin stain, Verhoeff-Van Gieson and etc.

II. Immunohistochemical (IHC) Staining

IHC is the application of immunologic principles and techniques for studying antigenic components of cells and tissues (3). Examples of diagnostically antigens that can be recognized include: endocrine cells and neuronal granules,
vesicles and peptides, cytokeratine and intermediate filaments, leukocyte markers, immunoglobulins, oncofetal antigens, proliferation markers, mucin core proteins and numerous enzymes (Figure 3) (4-11). Several procedures are available for IHC, however, currently the two most commonly used are prooxidase-antiprooxidase immune complex method and the biotin-avidin immunoenzymatic technique (12-16).

Figure 3. Immunohistochemical (IHC) Staining

III. Molecular unit
Amplification DNA technology has provided an important investigative tool in the field of GI pathology (17-19). Its value has been most obvious in explaining the pathogenesis of neoplastic disorders and uncovering the genetic basis of neoplasia, for which GI cancer served as an important model for solid tumors (20).

1. Polymerase chain reaction (PCR)
PCR is a simple, in vitro, chemical reaction that permits millions of copies of any specific DNA sequence to be generated within a few hours (21). This Technique is capable of amplifying a sequence 105-to 106 folds from nanogram amounts of template DNA using a heat-stable DNA polymerase and using an automated thermocycler (Figure 4).

Figure 4. Thermocycler machines.

In our laboratory, there are several researchers and gradient thermocyclers (Eppendorf Company, Germany) which enable us to easily detect the target DNA. PCR itself is consisted of different types, all experienced in each research center is including:

A. RT-PCR: Reverse Transcriptase PCR was developed to amplify RNA targets. In this process, complementary DNA (cDNA) is first produced from RNA targets by reverse transcription, and then the cDNA is amplified by PCR (22).

B. Nested PCR: Nested PCR was developed to increase both the sensitivity and specificity of PCR (23). It employs two pairs of amplification primers and two rounds of PCR for a single locus.

C. RAPD-PCR: Random Amplified Polymorphic DNA is a method of producing a biochemical fingerprinting of a particular species (24). Relationships between species may be
determined by comparing their unique fingerprint information.

D. ACR-PCR: In Amplification Created Restriction PCR a restriction site is created (25). In near future another PCR method will be utilized in this laboratory called “Real-Time PCR” that is a new method of PCR quantification which allows actually viewing the increment in the amount of DNA as it is amplified.

E. Real-Time PCR: describes methods by which the target amplification and detection steps occur simultaneously in the same tube (25). These methods require special thermal circlers with precision optics that can monitor the florescence emission from the sample wells (Figure 5).

2. RFLP (Restriction Fragment Length polymorphism)
Assays that utilize the sequence recognition property of restriction enzymes to demonstrate variations or polymorphisms in the DNA sequence of two samples are known as RFLP (26). Genomic DNA or RNA produced from a PCR reaction is digested with a restriction enzyme and the fragments analyzed by gel electrophoresis followed by band visualization or southern blot analysis with a site specific probe.

3. MSI (Micro Satellites Instability) study in HNPCC
Mutation in genes that repair damaged DNA cause regions called micro satellites to get longer or shorter, a phenomenon the scientists call micro satellite instability (MSI) (27-30). Testing for MSI helps us determining whether a person is likely to have a gene mutation, as described in hereditary non-polyposis colorectal cancer (31).

4. SSCP: Single Stranded Conformational Polymorphism
To scan a disease gene for any number of unknown mutations several screening techniques are available. SSCP is a process utilizing gel-electrophoresis to identify a segment of DNA containing a mutation. It can be used as the first step in mutation scanning (32).

5. DNA and RNA Extraction
In DNA extraction unit, serum, plasma and buffy coat will be isolated from the whole blood samples, then, the optimum density (DD) of the samples will be checked by spectrophotometer. One of the innovations of each GI center providing a DNA bank of patients with GI-related diseases, GI cancers, liver diseases, Celiac disease, IBD and etc. is require.

6. ELISA
Enzyme – linked immunosorbent assay is a useful and powerful method in estimating ng/ml to pg/ml ordered materials in the solution, such as serum, urine and culture super nations (33). ELISAS combine the specificity of antibodies with or antigens coupled to an easily-assayed enzyme (Figure 6).

7. Sequencing
In the sequencing unit, genotyping, determining mutations and drug resistance for viral hepatitis, small intestine and large intestine abnormalities
and other GI-related disorders and liver disorders will be evaluated by DNA sequencing in those research center that have accessibility to this machine (Figure 7).

In this part, agarose gel, polyacrylamide gel (PAGE) and SDS-PAGE (for protein detection) should be prepared and instruments such as horizontal and vertical electrophoresis tanks and the Gel-Documentation system are used for viewing and detection of PCR bands (figure 8).

8. Biochemistry section
Different biochemical tests such as liver function tests, CBC diff, Ferritin, Fe, Transferrin, etc. are performed in this section as.

9. Electrophoresis

10. FISH equipment
The absence of an elastin gene from a chromosome is detected by the FISH technique (34). FISH is an acronym for the technical expression “fluorescence in situ hybridization” (34). For example, a blood sample is taken from a patients and then treated with two specific colored markers that give off a "fluorescent" light when exposed to ultra-violet light.

One of the markers attaches to each of the two copies of chromosome number seven in a cell (35). When both copies of chromosome possess the elastin gene, an additional fluorescence of another color is seen attached at another location to each of the two chromosome 7s.
But, as is the case in over 95% of the Williams syndrome individuals tested, only one copy of chromosome seven, not two, will show the fluorescent spot for the elastin gene (36). For pictures illustrating chromosome seven and this test, check the article in the December, 1997 Scientific American magazine (http://www.ucd.ie/artspgs/langimp/williams.pdf). If the FISH test shows the elastin gene to be missing from one copy of chromosome seven, then the individual tested can be said to have Williams's syndrome.

Conclusion

Blood factors play a precarious role in diagnosis, assessing development, and in the classification of disease and phenotypes in clinical and research circumstances. The accuracy and reliability of the whole blood parameter analysis depends on identification and control or eradication of variables that may disturb these results. Clinical pathology deals with the use of laboratory methods (clinical chemistry, microbiology, hematology and molecular diagnostics) for the diagnosis and treatment of different human diseases are areas of active research and development. Pathology as a medical subject and an investigative scientific discipline, is concerned with understanding the essential nature of human GI disorders. We suggest to use a wide range of routine diagnostic services such as haematology, biochemistry, microbiology, cytology and pathology, as well as more specialised services like molecular methods in specific areas to investigate the prognosis, diagnosis, prevention and treatment of different disorders in the field of gastroenterology and liver diseases.

References


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