Immunohistochemistry

Immunohistochemistry (IHC) or immunocytochemistry is a histology method for detection of specific tissue or cell antigens based on antigen-antibody recognition. It exploits the specificity provided by the binding of an antibody to its antigen at light microscopic level. The history of IHC extends from 1940, when Long developed an immunofluorescence technique to detect corresponding antigens in sections of frozen tissue. However, only in the last decade has the method found general application in diagnostic surgical pathology. A number of technical developments in IHC have created sensitive detection systems. Avrameas, in 1972, published a suitable chromogenic substrate system, using horseradish peroxidase, which allowed visualization of the labeled antibody by light microscopy. One of the critical issues of the immunohistochemical techniques was related to the need for achieving greater sensitivity and specificity. Sensitivity improved with the development of multiple-step techniques to magnify the color signals and specificity with the development of the hybridoma technique, which enables the manufacture of large quantities of highly specific monoclonal antibodies. Sensitivity was also found to be negatively influenced by formalin fixation of the tissue. Formalin, universally used as fixative in histology, was found to “hide” tissue antigens, thus an alternative fixative was looked for. However, so far, a better fixative than formalin has not been found. Enzyme digestion was introduced by Huang and colleagues to “unmask” some antigens that have been altered by formalin fixation. However, the enzyme digestion method, while widely applied, did not improve IHC staining of the majority of antigens, in addition to being difficult to standardize. Difficulty of standardization provided incentive for developing a new technique with requirements more powerful, more widely applicable, and easier to use than the enzyme digestion method. In addition, the technique should enhance immunohistochemical staining of routinely formalin-fixed, paraffin-embedded tissue sections in a reproducible and reliable manner. Based on biochemical studies from Fraenkel-Conrat and coworkers, Shi and collaborators developed the antigen retrieval (AR) technique. The AR technique is a simple method that consists of heating routinely processed formalin-fixed paraffin embedded sections to high temperatures before IHC staining. Since then, the intensity of IHC staining was dramatically improved as was demonstrated by numerous reports in the literature. Worldwide application of AR-IHC in anatomic pathology has validated the feasibility of AR-IHC and expanded its use in molecular morphology. The Histology laboratory of our department currently offers IHC to detect more than 60 tissue antigens to help in the molecular characterization of pediatric diseases.
**Lactic Acidosis: D versus L**

Generally speaking, lactate or lactic acidosis refers to L-lactate. D-lactate is a different entity. Both D- and L-lactate are primarily formed in the process of anaerobic carbohydrate metabolism. However, in humans, only L-lactate, NOT D-lactate, is produced. When D-lactate is detected in the human body, it is a product of bacterial carbohydrate metabolism. It is not uncommon to confuse D-lactate with L-lactate. Usually, L-lactate (commonly just referred to as lactate) is a needed test and is ordered correctly. However, sometimes D-lactate is ordered when the actual test needed is L-lactate, or vice versa. The routine test in most hospital laboratories, including the CMH Laboratory, is L-lactate.

Strenuous exercise can produce more than a tenfold increase in plasma L-lactate within several seconds. L-lactate returns to baseline quickly after cessation of exercise. Even hand-clenching and prolonged use of a tourniquet during blood draw result in increased L-lactate. Pathological causes of L-lactic acidosis are divided into two types. Type A is due to tissue hypoxia and is more common. Type B is due to acquired or congenital diseases or drugs or toxins. Causes of these two types of L-lactic acidosis are given below.

**Type A (tissue hypoxia apparent or probable):**
- Severe hypoxia
- Severe anemia
- Shock
- Cardiac failure

**Type B (tissue hypoxia not apparent or unlikely)** is further divided into three types:
- Type B1: Due to acquired diseases, such as diabetes mellitus, liver failure, convulsion, and malignancy.
- Type B2: Due to drugs or toxins, such as biguanides, metformin, ethanol, methanol, ethylene glycol, propylene glycol, isoniazid, and salicylate.
- Type B3: Due to inborn error of metabolism, such as glucose-6-phosphatase deficiency, fructose 1, 6-phosphatase deficiency, mitochondrial disorders, Type-I glycogen storage disease, pyruvate dehydrogenase or carboxylase deficiency, fatty acids oxidation defects, defects in biotin metabolism (biotinidase deficiency, holocarboxylase deficiency), and some organic acidurias (HMG-CoA lyase deficiency, propionic acidemia, methylmalonic acidemia).

Type B lactic acidosis can be accompanied by Type A due to hypoperfusion of tissues.

D-lactic acidosis is generally caused by carbohydrate metabolism in the gastrointestinal tract by gram-positive organisms, such as *Lactobacillus* species, *Streptococcus bovis*, *Bifidobacterium* species, and *Eubacterium* species. The causes include bacterial intestinal overgrowth, intestinal blind loops, and short-bowel syndrome. Malabsorption of carbohydrates or excessive load of carbohydrates may also result in D-lactic acidosis. A patient may develop severe acidosis, and plasma L-lactate (as measured by commonly used methods), 3-hydroxybutyrate, and acetoacetate levels may be completely normal. However, in D-lactic acidosis, urine organic acid analysis shows the presence of a large amount of lactic acid in the urine. This is because the urine organic acid analysis measures the total (D and L) lactate and does not differentiate between D- and L-lactate. Therefore, in patients with normal plasma L-lactate but high urine total lactate, D-lactic acidosis should be suspected. In these patients, a specific test for D-lactate should be performed.