

## Editorial

### Contents

|                    |    |
|--------------------|----|
| ■ Editorial        | 1  |
| ■ Mini review      | 2  |
| ■ Current Trends   | 8  |
| ■ In Profile       | 11 |
| ■ Relaxed Mood     | 12 |
| ■ Bug of the Month | 13 |
| ■ Did you Know     | 14 |
| ■ Best Practices   | 16 |
| ■ In Focus         | 18 |

Well to Jump start with this issue we have the **Mini Review Section** – In order to visualize biological structures, representative samples are specially prepared, which typically involves preservation and subsequent coloration (staining) prior to microscopic examination.

Stains, which in a sense can be considered to be a category of histologic probes, are commonly used in histology to make the structure of tissues more easily visualized than in unstained preparations, and to reveal chemical and physical differences in the various cellular and tissue components. The majority of biological stains are synthetic organic dyes, often borrowed or modified from the textile industry.

**Current Trends Section** - Pure water, also known as purified water, is water from a source that has removed all impurities. Distilled water is the most common form of pure water. Pure water can be purified by carbon filtration, micro-porous filtration and ultraviolet oxidation. Some places use combination of purification processes. There are many different types of water purifiers available in market. Water purifiers range from simple water filters to advanced purifiers using membrane technology for water filtration followed by disinfection with UV lamp filters.

**In Profile** - Paul Ehrlich prophesied the role of modern-day pharmaceutical research, predicting that chemists in their laboratories would soon be able to produce substances that would seek out specific disease-causing agents. He called these substances “magic bullets.” Ehrlich himself met with signal successes in the emerging fields of serum antitoxins and chemotherapy.

**Bug of the Month** - Yersiniosis is a relatively uncommon infection contracted through the consumption of undercooked meat products (especially pork), unpasteurized milk, or contaminated water. Of the three main types of yersiniosis that affect people, *Yersinia enterocolitica* (bacteria that thrive in cooler temperatures) are responsible for most infections in the United States. The bacteria can infect the digestive tracts of humans, cats, dogs, pigs, cattle, and goats. People can contract it by eating or handling contaminated foods (such as raw or undercooked meat) or by drinking untreated water or unpasteurized milk that contain the bacteria.

**Did You Know?** - Q fever, also called query fever, is a bacterial infection caused by the bacteria *Coxiella burnetii*. The bacteria are most commonly found in cattle, sheep, and goats around the world. Humans typically get Q fever when they breathe in dust that was contaminated by infected animals. Farmers, veterinarians, and people who work with these animals in labs are at the highest risk of being infected. The highest amounts of bacteria are found in the "birth products" (placenta, amniotic fluid) of infected animals.

**Best Practices** - Foot pain is one of the adverse side effects of diabetes. For this reason, foot care is integral to diabetic care and management. Unattended feet can become problematic and painful, sometimes, leading to amputation as well. Depending on the extent of pain and damage, medications can be given although the first line of action is to control the blood glucose level.

Your inputs are a valuable contribution towards making this Journal more successful & looking forward for your continuous support & appreciation.

# Biological Stains and its Applications (Issue-I)

In order to visualize biological structures, representative samples are specially prepared, which typically involves preservation and subsequent coloration (staining) prior to microscopic examination.

Stains, which in a sense can be considered to be a category of histologic probes, are commonly used in histology to make the structure of tissues more easily visualized than in unstained preparations, and to reveal chemical and physical differences in the various cellular and tissue components.

The majority of biological stains are synthetic organic dyes, often borrowed or modified from the textile industry. An older group of stains consists of dyes obtained from natural sources (e.g., hematoxylin from the bark of Logwood trees; carmine, from the bodies of certain insects). Natural dyes were used almost exclusively until the synthetic dyes were developed in the mid-19th century. In addition various metals are used alone or in combination with certain dyes to enhance their action. For example aluminum, iron, or tungsten, are used in combination with oxidation products of hematoxylin to increase the effectiveness of staining specific cellular structures. On the other hand, silver and osmium tetroxide, which have an innate affinity for certain cell and tissue components can be used as stains in their own right. Although several thousand stain and other procedures for coloring cells and tissues are in usage, some of the most common are summarized in this issue.

Histology [Gr. *histos* =web, tissue + *logos* =study of.] is the study of the structure and function of cells, tissues, and organs of the body at the microscopic level. Hence, it is often referred to as Cell, Tissue & Organ Biology. In the past, it has also been called microscopic anatomy.

Tissues [Fr. *tissu* =fabric, texture; from L. *texo* =to weave] are best defined as structures of cells and their associated extracellular substances/matrix (ECM), which are specialized to carry out particular functions. Four primary tissues or cellular structures are defined: epithelium, connective tissue, muscle, and nervous tissue. Organs are composites of these primary tissues and based upon their composite functions are arranged into systems: nervous, integument, cardiovascular, lymphoid, alimentary, respiratory, urinary, endocrine glands, and genital systems.

## Tissue Preparation & Processing

Various technical steps must be carried out in order to achieve optimally prepared specimens for microscopic examination. Unfortunately, at each of these steps the introduction of artificial structures, which are commonly known as histologic artifacts, can be introduced by the processing or processor. These detractors from normal tissue architecture can be many and include tissue folds; accumulation of external dirt or other debris; cracks in cells and tissues; marks, chatter or gouges in tissues that are introduced by cutting implements; and even such subtle changes as tissue shrinkage. Learning to recognize these artifacts is usually easy and straight forward, but some may present a challenge. Only a brief overview of tissue preparation and processing will be presented here.

## Fixation

1. *Definition and mechanism of action.* Fixation is defined as the rapid preservation of tissue components in order to arrest cellular

processes and to maintain, as close as possible, a resemblance to the living condition. In histology, a fixative is typically a chemical agent, which may consist of a simple solution (e.g., 10% formalin: formaldehyde dissolved in an aqueous or buffered medium) or more complex solutions (e.g., Bouin fixative: picric acid, acetic acid, formalin). The end result is a crosslinking and denaturation of tissue components, particularly proteins. Equally important, fixation also resists tissue degradation by endogenous (enzymatic autolysis) or exogenous (bacterial action) mechanisms. While many fixatives are aqueous, some are also alcohol-based.

## 2. Fixation procedures are generally of two different types: Immersion and Perfusion.

a. *Immersion fixation*, which is the most routinely used method, is performed by placing small pieces of tissue into a relatively large volume of fixative. This ratio is chosen to facilitate an interaction between the two that may result in as rapid and complete fixation as this method will allow. The rate of diffusion, which is dependent on the properties of both the fixative and tissue type, is obviously a limiting factor for optimal preservation.

b. *Perfusion fixation*, which is much more complex and often difficult to perform depends on the introduction of fixative into tissues via the vasculature. Nevertheless, this method has the greatest potential for rapid and fine preservation of histologic architecture.

## Dehydration and clearing

Since histologic techniques are either performed in an aqueous or organic medium, both dehydration and rehydration sequences must be used to move tissue specimens between phases. If a fixative is aqueous and the embedment is water insoluble (e.g., paraffin wax), then a dehydration sequence is used to gradually introduce an organic phase that will be compatible with the embedding medium. Typically, this sequence is a graded series of ethanol's (e.g., 50, 70, 95, 100%). Once absolute ethanol is achieved, the tissue is placed in an intermediate organic solvent that is miscible with both the alcohol and the wax (e.g., xylene, toluene, benzene, or other less toxic synthetics). Since opaque tissue specimens became clear when placed in some of these agents, they were collectively referred to as "clearing agents" or "clearers", even though not all may result in tissue "clearing".

## Infiltration and embedding

In order for the paraffin wax to mix with and replace the clearing agent within the tissue specimen, the wax is heated to its melting temperature. The specimen is then placed in several changes of molten wax, often under vacuum, in order to replace the clearing agent and completely infiltrate the tissue with the wax embedding medium. Since the paraffin melting temperature is significantly elevated over room and body temperatures, various artifacts (e.g., shrinkage) are introduced by this method. When infiltration is complete, the tissue is embedded in fresh wax, which is then allowed to harden at reduced temperatures within a special embedding mold.

## Sectioning and mounting

Embedded tissue is removed from its mold, trimmed, mounted and secured on a cutting instrument called the *microtome*. This

device typically advances the tissue in micrometer increments across a cutting knife so that thin slices of tissue (typically 2-10  $\mu\text{m}$  in thickness) are obtained. These tissue sections are then mounted on glass slides for subsequent coloration or other processing.

### Staining and histochemistry

These are procedures for visualizing tissue components by means of various coloring techniques. These procedures take advantage of the intra- and extra-cellular chemistry of the tissues. Details of some important methods are presented in the section on Dye-Cellular Interactions below.

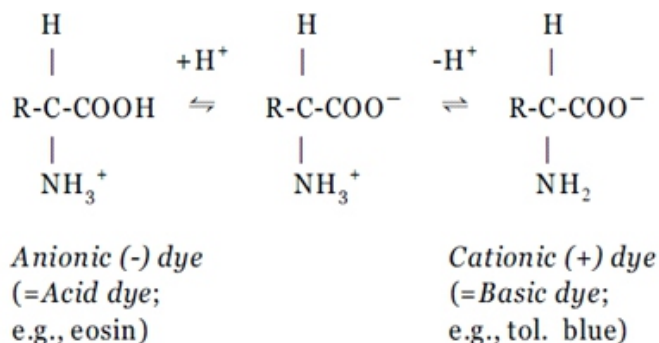
### Coverslipping

Following staining procedures, tissue sections affixed to slides are typically covered by a thin glass disk or plate (square or rectangle) called a coverslip or cover glass. These coverings not only protect tissue specimens from the environment, but they also facilitate microscopic examination, micrography, and other manipulations.

## DYE-CELLULAR INTERACTIONS

### Basophilia and acidophilia

Proteins are the substances that contribute in large measure to the stainability of tissue sections. Since proteins are amphoteric, they possess both acidic and basic groups. At the isoelectric point ( $pI$ , see Fig. 1), the net charge is zero and minimal dye binding occurs. If staining takes place above the isoelectric point of a given protein, its acidic groups (phosphate, carboxyl, or sulfate) will be negatively charged (Fig. 1) and can combine with a positively charged cationic dye such as methylene blue.



**Fig1.** Schematic representation of dye-protein interactions. At  $pI$  of protein (center), negligible binding of charged dyes occurs. Below  $pI$  (left), protein binds acid(=anionic) dyes; above  $pI$  (right), protein binds basic(=cationic) dyes. At physiologic pH, specific proteins may exhibit net (+) or net (-) charges and are therefore characterized as acidophilic (=affinity for acid dyes) or basophilic (=affinity for basic dyes), respectively.

Since the dye is forming a salt linkage with the residue of an acidic group, it is termed a basic dye and the substance stained is termed *basophilic* (base-loving). Conversely, basic groups (primarily amino) are positively charged (Fig. 1) so that at a pH below their isoelectric point they will bind anionic dyes such as eosin. Such dyes are therefore called acid dyes and the substance stained is termed acidophilic (acid-loving), or eosinophilic in the case of cell components that stain with eosin.

Basophilic substances are of considerable biological importance. The phosphate grouping is abundant in nucleic acids (in both ribose and deoxyribose forms). Thus, nuclei, nucleoli, and

ribosomes (free and ER-bound) are basophilic. Negatively charged carboxyl and sulfate groups are found abundantly in glycosaminoglycans (GAG's), which are linear sugar polymers that are associated with core and linkage proteins to form complex branched proteoglycans. The weakest binding (weakest basophilia) is by phosphates; the strongest is by sulfates. Likewise, a number of acidophilic substances (positively charged substances) are also important: examples include collagen, keratin, hemoglobin, and muscle proteins.

### Basic dyes and acid dyes

1. **Synthetic dyes.** The commonly used aniline dyes are neutral salts. Thus, the colored portion, upon dissociation, may be positively or negatively charged, depending upon the dye. Methylene blue and toluidine blue exist as chlorides that dissociate in solution to yield a positive charge on the color bearing portion. Thus these dyes are cationic (basic) dyes. Eosin occurs as the sodium salt, and the colored moiety is negatively charged upon dissociation. Thus, eosin is an anionic(acid) dye. Other commonly used anionic dyes are aniline blue, orange G, picric acid, and acid fuchsin. (refer Table 1. for summary of important basic and acid dyes).

2. **Natural dyes.** Certain stains are obtained from natural sources and differ from the synthetic dyes in their general properties. Their chemistry is not as well understood as that of the synthetics. Perhaps the most important natural dye is hematoxylin. Hematoxylin is used as a basic dye but actually is not a dye as such, and must be oxidized ("ripened") to hematein to be a stain. Furthermore, the hematein component of this complex mixture is not a basic dye at all. Rather a mordant or "go-between" of a metallic nature must be used with hematoxylin in order for this mixture to behave as a basic dye. [N.B.: Remember that hematoxylin per se is not a "basic" dye and only stains (basophilic) tissues due to the affinity of its hematein component for metals.] Nevertheless, despite this limitation, hematoxylin is commonly referred to as a basic dye because the cell and tissue structures that are staining are basophilic. The familiar H&E preparation employs eosin as a counter stain. Eosin stains those structures that are positively charged and have no affinity for a metal mordant; recall that such structures are said to be "eosinophilic" or "acidophilic".

### Metachromasia

An interesting property exhibited by certain dyes (usually basic varieties; e.g., toluidine blue) is called metachromasia. This phenomenon is described as the ability of a dye to change from the orthochromatic or usual color (blue in the case of toluidine blue) to a metachromatic color (purple in this case) in the presence of highly acidic (polyanionic) substances. This chromatic shift is explained as an effect on dye color due to interactions between the closely adjacent, abundant dye molecules bound by the strongly acidic molecules. In dilute solution, toluidine blue is monomeric and blue in color. In concentrated solution, dye molecules polymerize and thereby exhibit the metachromatic color shift. Thus, the effect of polyanions, due to their high negative charge density, is to functionally polymerize the toluidine blue molecules in tissues. As a result, the metachromatic staining reaction is a great predictor of tissue polyanions. Important examples of cellular structures and tissues that exhibit this property are mast cell granules containing heparin and proteoglycan, and cartilage ECM containing chondroitin sulfate proteoglycans. Other basic

dyes that exhibit metachromasia are methylene blue, thionin, and the azures. Metachromatic staining with the acid dye Biebrich Scarlet has also been reported (Spicer, *SS Exp Cell Res* 28:480, 1962).

### Romanowsky dyes

1. *Background.* The Russian physician Dimitri Romanowsky (1861-1921) was apparently the first investigator to combine eosin and methylene blue and use this mixture for staining blood in his studies on malarial parasites (Romanowsky, *DL Imp MedMil Acad, Dissert No 38, St Petersburg, 1891*). Thus, present day mixtures of acid (eosin) and basic (methylene blue, azures, etc) dyes that are applied simultaneously are referred to as modified Romanowsky stains. In these procedures, the pH must be carefully controlled at or near neutrality.

2. *Usage and examples.* These mixtures are most commonly used for blood or bone marrow smears and produce elegant differential staining of the leukocytes (white blood cells). These stains are also exquisite indicators of cytoplasmic basophilia. Wright stain and Giemsa stain are common examples of Romanowsky dye mixtures.

3. *Properties.* Chemically, these mixtures have been characterized as eosinates of methylene blue and/or azure derivatives of methylene blue. The combined stain had properties different from the stains used alone which is the true nature of the mixture and its mechanism of action.

4. *Mechanism.* The staining mechanism has been elucidated to include 3 fundamental processes (Horobin, RW & Walter KJ *Histochemistry* 86:331-336, 1987): 1) orthochromasia (cellular components stained either pink/red due to eosin or blue due to basic dye component), 2) polychromasia (layered staining with both dyes), and 3) metachromasia (color shift of basic dye from blue to violet-purple due to high concentrations of polyanions). See **Table 1** for examples of blood smear components that are orthochromatic (O), polychromatic (P), and metachromatic (M).

### Staining due to physical properties of dyes

**Vital staining.** Vital stains are employed to color certain components within living organisms without doing harm to the vital tissues. For example, trypan blue and India ink form colloidal suspensions in water. Since these large particulates will not diffuse into cells, they will be cleared from the body by the phagocytic activity of macrophages, and are thus excellent markers for members of the mononuclear phagocyte system (MPS). Supravital staining is a variant of this procedure in which these dyes are applied to living tissues that have first been removed from the organism by, for example, *biopsy*.

**Lipid staining.** Lipid colorants such as Sudan black and Oil Red O are soluble in lipid and are used to demonstrate fat in tissues if the lipid has not been extracted; usually these procedures are performed on frozen sections.

**Schiff reaction.** Some dyes possess other chemical characteristics that are useful in revealing certain cell and tissue constituents. One of the more useful dyes in this regard is basic fuchsin, a red dye in solution that becomes colorless when reduced by the addition of acid and an excess of SO<sub>2</sub>. The German chemist Hugo Schiff (1834-1915) was the first to recognize that this colorless solution could be recolored to bright magenta by the addition of aldehydes (Schiff, H *Justus Liebigs Ann Chem* 140:92-137, 1866). Thus this solution, which is now called the *Schiff reagent*, is widely used in organic chemistry and

histochemistry to detect aldehydes by forming the stable red-magenta reaction product. In histology and pathology, this reaction has not only been utilized in a number of histochemical techniques, but has been of particular significance in studies involving the localization of DNA and complex carbohydrates.

**Feulgen reaction.** An important example of the use of the Schiff reagent is in the Feulgen technique for demonstration of deoxyribo nucleic acid (DNA). Also known as the nuclear reaction, this technique was developed by the German biochemist Robert Feulgen (Feulgen, *R Z Physiol Chem* 92:154-158, 1914; Feulgen, R & Rossenbeck, *H Z Physiol Chem* 135:203-248, 1924). In this two-step procedure, DNA is first hydrolyzed in weak HCl to yield aldehydes, and then the Schiff reagent is used to detect the resulting aldehyde groups in the nucleus. Since this technique is specific for DNA and not RNA, it is an important procedure for discriminating between the two at the cell and tissue level. The specificity of this reaction results from the first step. Since this reaction "is both specific and stoichiometric for DNA, it has become the most important means of staining nuclear DNA for densitometric quantification" (Hardie, DC et al *J Histochem Cytochem* 50:735-749, 2002).

**Periodic acid-Schiff (PAS).** This technique, which is based on the independent work of several investigators (Bauer, *H Z Mikrosk Anat Forsch* 33:143-160, 1933; Lillie, RD *J Lab Clin Med* 32:910-912, 1947; McManus, *JFA Nature* 158:202, 1946; Hotchkiss, RD *Archiv Biochem* 16:131-141, 1948), is widely used for the demonstration of vicinal-glycol moieties in carbohydrates. In this procedure, the tissue is first treated with periodic acid. The 1-2 glycol linkages, if present, are then converted (oxidized) to aldehyde groups, which subsequently combine with the clear Schiff reagent to form the colored (magenta) reaction product. Thus, this reaction is also a two step procedure. The specificity results from the periodic acid step.

### Metals

Several metals are used in histologic techniques.

1. **Silver impregnation methods** are employed to demonstrate the Golgi apparatus, reticular fibers, neuro fibrils and some other structures. The results of these "stains" depend on the method used.

2. **Osmic acid (osmium tetroxide)** is useful for the demonstration of certain lipids because unsaturated fatty acids reduce it to a black compound. Osmic acid is often used to demonstrate the Golgi apparatus. The reaction depends on a lengthy impregnation procedure. Osmium is also used as afixative/stain for electron microscopy due to its ability to preserve membrane lipids and also to give them some contrast.

3. **Gold chloride** is used in neurology to demonstrate nerve endings and astrocytes; it has also been used to demonstrate Langerhans cells in the skin (Ferreira-Marques, *J Arch Dermatol Syphilol* 193:191-250, 1951; Breathnach, *AS Int Rev Cytol* 18:1-27, 1965).

### Special stains

Whereas the most commonly used staining combination is undoubtedly H&E (it is perhaps used more than all other staining combinations put together), many other staining combinations have been employed to emphasize specific tissue characteristics.

**1. Connective tissue stains.** A wide variety of special stains to enhance connective tissue elements have been developed and consist of a battery of several different colored/shaded acid and basic dyes; e.g., Masson trichrome (Masson, *P Am J Pathol* 4:181-211, 1928), Mallory triple (Mallory, *FBPathological Technique WB Saunders, Philadelphia, 1938*), Movatpentachrome (Movat, *HZ Archiv Pathol* 60:289-295, 1955).

Several images in the database collection exhibit examples of these “polychromes”. Although the chemistry of dye binding in many of these techniques is obscure (in some, for example, there are three dyes that are all acid dyes), they are excellent in distinguishing important elements. For example, it is sometimes difficult to distinguish cytoplasm of cells amidst extracellular collagen after H&E and, conversely, to distinguish collagen from cellular material such as in nerves and tendons. The connective tissue stains distinguish these differences clearly, no matter which one of several stains is used. A common stain of this group is the Masson procedure. The identifying feature when seeing such a stain is that collagen fibers of connective tissue are stained either blue (aniline blue) or green (fastgreen). Cytoplasm, in contrast, will be red-orange to red-lilac.

**2. Eosin-methylene blue (eosin-azure).** Although not as common, several examples of these mixtures are presented in the database collections. Dilute solutions of a basic stain (methylene blue or azure) and an acid stain (eosin) are used in a buffer, so that cytoplasmic basophilia is much more evident than with H&E. This combination yields similar results to those of the stains for blood smears (Wright or Giemsa). However, here the techniques are adapted to sectioned material rather than for smears.

### Special probes

Labeling components of cells and tissues using special probes for enhanced specificity are valuable tools with important applications for biomedical research and clinical medicine.

#### 1. Immunocyto (histo)chemistry.

Histochemistry (cytochemistry) encompasses a very large family of valuable techniques that yield much greater detail than normal staining methods when applied to the localization and distribution of the component parts of cells and tissues. Immunocytochemistry (ICC; IHC), which uses the principles of immunology to locate and identify specific antigens with labeled antibodies, was recognized as having even greater power than traditional histochemical reactions. The first studies used a fluorescent dye as the label or chromogen [Gr. *chroma* = color; *gen* = suffix denoting “precursor of”], which was coupled to antibodies in order to identify a certain antigen in tissue sections (Coons, AH et al. *Proc Soc Exp Biol Med* 47:200-202, 1941).

**a. Methods.** Although there are now many variants on IHC (direct vs indirect; labeled vs unlabeled antibody; enzyme-conjugated vs fluorescent; etc), the basic theme involves the following steps: 1) producing antibodies to specific antigens; 2) using these antibodies to localize antigens of interest in cells and tissues; 3) coupling the antibody-antigen complexes with chromogens in order to form conjugates in tissue sections; 4) developing and/or detecting the chromogens in order to visualize the existence and location of the desired antigen. To amplify chromogen signals (increase sensitivity) in these procedures, it is now common to use a primary antibody for the antigen of interest and either secondary or tertiary antibody-chromogen conjugates,

which bind to the primary antibody-antigen complex or a secondary bridge(link) antibody, respectively. Other high affinity bridge techniques (e.g., avidin-biotin) are also used.

**b. Applications.** In addition to the great advances in detection, localization, and life histories of specific markers either in or on cells in health and disease, virtually every aspect of biomedical research and diagnostic medicine has benefitted from these techniques. For example, in 2000 it was estimated that almost every diagnostic pathology laboratory routinely uses more than 100 antibodies for the detection of specific antigens by these methods (Coleman *R Acta Histochem* 102:5-14, 2000).

**2. Radio autography (autoradiography\*)** has been an important technical method for localizing radio labeled substances and studying dynamic events (e.g., secretion pathways, cell turnover) in tissue sections. It was developed more than 50 years ago for light microscopy (Belanger LF & Leblond CP *Endocrinology* 39:386-400, 1946) and subsequently adapted for electron microscopy (Salpeter M J *Cell Biol* 32:379-389, 1967). Although these applications have made significant contributions to understanding fundamental biologic processes, their use has declined somewhat in recent years partly due to concerns and regulations about radioactive isotopes, and also due to the development of some alternative methods (e.g., BrdU labeling; see below).

**a. Methods.** “Precursor” molecules (e.g., nucleotides, amino acids, sugars) for larger “products” (nucleic acids, proteins, complex carbohydrates) or certain finished “products” (e.g., hormones) are labeled with a radioactive isotope (C, H, <sup>14</sup>S, <sup>125</sup>I). Radiolabeled molecules [e.g., tritiated thymidine for labeling DNA (Taylor JH et al *Proc Natl Acad Sci* 43:122-128, 1957)] are injected into the body and tissue samples are subsequently removed at various time intervals (in order to observe dynamic activity over time). Tissue sections, which have incorporated the labeled molecules, can be prepared for either light or electron microscopy. They are then covered with a photographic (silver halide) emulsion and placed in light-proof containers. The emulsion, bombarded by the radioactive emissions, becomes “exposed” while stored in the dark. Development of the emulsion (as in photography) results in the appearance of black silver grains, which are now localized over the sites of incorporated label. Whereas film exposure in photography is a function of shutter speed and aperture settings, optimal “exposure” in radioautography is achieved by keeping a series of specimens in the dark for various time intervals before development.

**b. Applications.** Radioautography has played important roles in contributing to our understanding of many fundamental and dynamic processes of cells and tissues including DNA synthesis and cell division, protein synthesis and secretion, function of the Golgi apparatus, mechanisms of exocytosis and endocytosis, and hormone binding to receptors and target cells.

**3. Bromodeoxyuridine detection.** Cell proliferation has been studied in many tissues by a variety of techniques such as counting mitotic figures and radioautography following [<sup>3</sup>H] thymidine incorporation into DNA as mentioned above. In 1982, Gratzner et al. described a sensitive, non isotopic, monoclonal antibody method for detecting DNA replication in single cells (Gratzner HG *Science* 218:474-475, 1982).

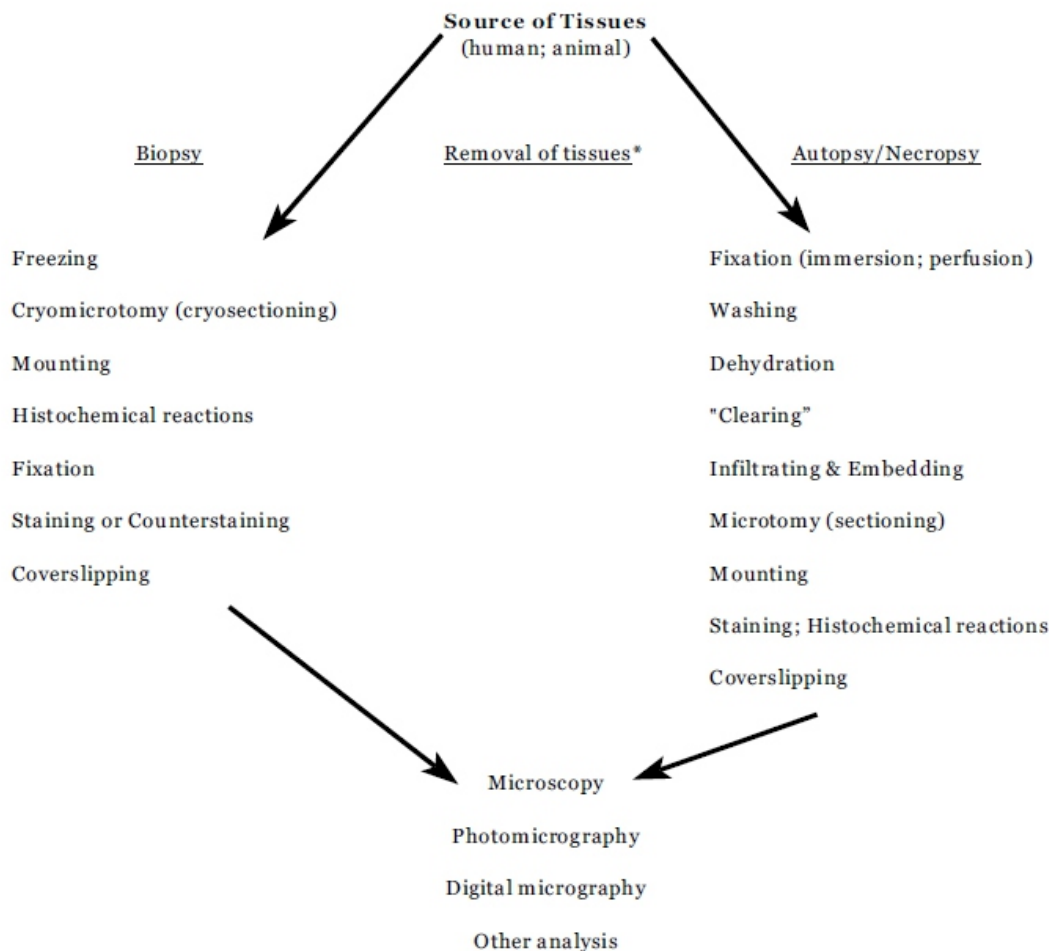
**a. Methods.** In this immunohistochemical procedure, the thymidine analog 5-bromodeoxyuridine (BrdU) - which is incorporated into nuclear DNA during S-phase before mitosis – is localized in tissue sections and reveals cells that have recently undergone cell division. Although first described using an immunofluorescent chromogen, BrdU IHC like other IHC procedures can use a variety of chromogens.

**b. Applications.** This technique is a sensitive method that is widely used for detecting DNA replication *in vitro* and *in situ*.

**4. In situ hybridization.** It has long been recognized that double-stranded DNA in solution can be denatured (“melted”) into single strands by heat or elevation of pH. Reversal of these manipulations allows the single strands to be recombined (“annealed”). This process of molecular hybridization is highly specific since only complementary strands will combine. Thus, hybrids between DNA-DNA, DNA-RNA, and RNA-RNA are possible.

**a. Methods.** These hybridization techniques were then applied *in situ* to cytological preparations on microscope slides to permit detection and localization of RNA-DNA hybrids (Gall JG & Pardue ML. Proc Natl AcadSci USA 63:378-383, 1969) and DNA-DNA hybrids (Pardue ML & Gall JG. Proc Natl AcadSci USA 64:600-604, 1969) at the cell and tissue levels. These studies used radioactive (tritium labeled) probes, which were detected in the complementary hybrids by radioautography. Subsequently, non-isotopic techniques were employed using enzyme-labeled (e.g., peroxidase antiperoxidase) or fluorescent-labeled probes, the latter of which is referred to as FISH (Fluorescence In Situ Hybridization). EM techniques have also been developed.

**b. Applications.** These techniques have had great utility in detecting nucleic acids in their cellular environment. Extremely useful in both research and clinical diagnosis, important applications include time course and location (differential gene expression) of mRNA transcripts, location of genes to specific chromosomes, recognizing chromosome abnormalities and pathologies, and diagnosis of genetic diseases.



**Figure 2.** Flowchart for the Preparation of Tissues for Microscopic Analysis

\*Although the path at the left for biopsy material may be more typical and vice versa, either paths can be used for biopsy and autopsy/necropsy specimens.

**Table 1. Important Stains and Histochemical Reactions (HR\*).**

| Reagent  | Type                                   | Color   | Tissue components visualized  |
|--|--|---|---|
| Hematoxylin  | “basic” dye                            | blue to bl-black                                | Nuclei, cytoplasmic and extracellular anionic substances (e.g., RER, proteoglycans)   |
| Iron hematoxylin   | “basic” dye                            | bl-black to black                               | Nuclei, mitochondria, muscle striations, RBCs, meiotic chromosomes.   |
| PTAH   | >”basic” dye                           | bl-black  | terminal bars, intercalated disks.  |
| Toluidine blue<br>Methylene blue<br>Azures                     | basic dyes                             | blue  | cellular & EC anions; metachromatic (purple) with high density polyanions(e.g., mast cell granules, basophil granules, cartilage ECM)   |
| Eosin<br>Orange G<br>Picric acid<br>Fast green<br>Aniline blue | acid dyes                              | pink-<br>red orange yellow<br>green<br>blue     | cellular and EC cationic substances (e.g., Hb, collagen, muscle proteins, keratin tonofibrils)  |
| Alcian blue  | basic dye                              | bl-green  | high density polyanions (e.g., GAG’s)   |
| PAS  | HR*                                    | magenta   | vic-glycols (e.g., glycogen, mucins, GAG’s)   |
| AB-PAS   | dye/HR*                                | bl-green<br>magenta royal<br>blue               | anions (e.g., acidic mucins w/out vic-glycols)<br>vic-glycols (e.g., mucins w/out acidic groups)<br>anions and vic-glycols (e.g., mucins w/ both)                                 |
| Feulgen reaction   | HR*                                    | magenta   | nuclear DNA (mitochondrial DNA in too low concentration to be detected)   |
| Silver   | metal                                  | black   | Golgi apparatus, reticular fibers, neurofibrils(argyrophilia: affinity for silver)  |
| Romanowsky dyes<br>Wright<br>Giemsa                            | mixture of acid and basicdyes          | dk purple<br><br>blue<br>lt purple pink-<br>red | leukocyte nuclei (P), basophil granules(M), platelet granulomere (P), cytoplasm of agranulocytes, esp. lymphs (O) azurophilic granules (P) RBC cytoplasm, eosinophil granules (O) |
| Vital stains<br>Trypan blue<br>India ink                       | nontoxic colloidal suspensions         | blue black                                      | phagocytic vacuoles in cells of mono nuclear phagocyte system, e.g., CT macrophages   |
| Lipid stains<br>Sudan black<br>Oil red O<br>Osmium tetroxide   | lipid-soluble particles<br>metal oxide | black<br>red<br>black                           | in situ lipid droplets containing triglycerides, sterols, etc<br>unsaturated lipids, phospholipids  |
| Elastin stains<br>Orcein<br>Resorcinfuchsin<br>Verhoeff        | -                                      | red purple black                                | elastin, fibers and sheets  |
| Masson trichrome   | polychrome (CT stain)                  | bl-black red<br>green blue                      | nuclei<br>cytoplasm, muscle<br>collagen, mucus  |

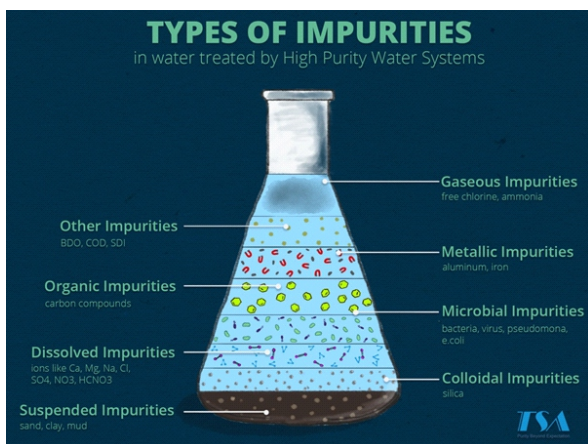
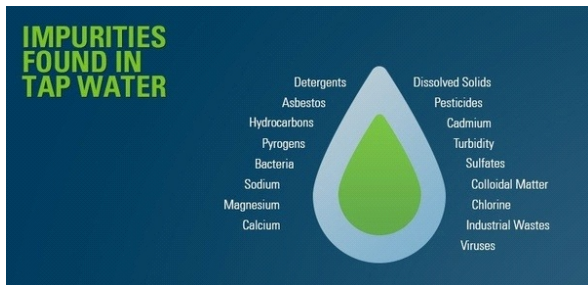
\*HR =histochemical reaction

# Different types of water purification

Pure water, also known as purified water, is water from a source that has removed all impurities. Distilled water is the most common form of pure water. Pure water can be purified by carbon filtration, micro-porous filtration and ultraviolet oxidation. Some places use combination of purification processes.

## Various types of systems

- Distillation
- Filtration
- water softening
- reverse osmosis
- ultraviolet radiation
- carbon treatment
- Deionization
- chemical disinfection



There are many different types of water purifiers available in market. Water purifiers range from simple water filters to advanced purifiers using membrane technology for water filtration followed by disinfection with UV lamp filters.

Here are the common water purification methods used for filtering domestic water:

### Membrane technology purification

#### RO purification:

Reverse osmosis is the most widely used water purification method. RO uses membrane technology to remove dissolved

salts, impurities and germs from water. The semipermeable membrane separates germs and dissolved chemicals from water. The membrane has very fine pores that allows only water to pass through it leaving behind all the harmful chemical, dissolved salts and microbes suspended in water (size of RO membrane pore is approximately .0005 micron which is slightly larger than the size of water molecule).

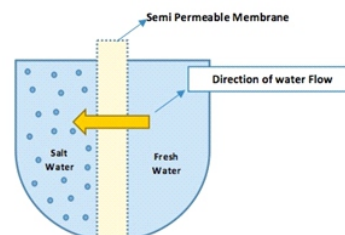
The drawback of RO is that it alters the taste of water and may remove some essential mineral from water. There are certain brands of RO which claim to retain the essential minerals in water. The semipermeable membrane require regular care and maintenance. To know more about RO process refer to the basic functionality of your RO in a very simple way.

RO stands for Reverse Osmosis which simply means that it is the opposite of a process called Osmosis. So let's first understand the Osmosis Process.

### What is Osmosis?

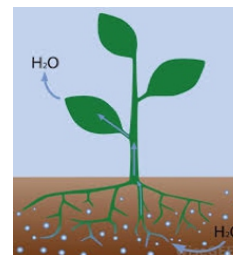
There is a natural tendency of fluids or solvents to pass from a lower concentrated solution to a higher concentrated solution through a semipermeable membrane or film. Semipermeable membrane is a film that allows only the fluid or solvent to pass but not the salts and impurities dissolved in it.

In the below diagram, fresh water has lower salt concentration compared to the salty water on the other side of the membrane. And therefore fresh water flows towards salt water (i.e. from lower concentration towards higher concentration)



Osmosis process explained

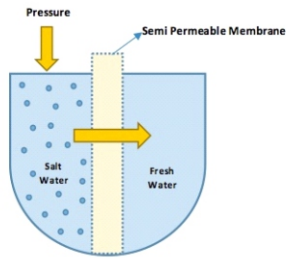
The simplest example is the passage of water from soil (soil has a higher concentration of rich salts and minerals) to the roots of plant.



### What is RO (Reverse Osmosis?)

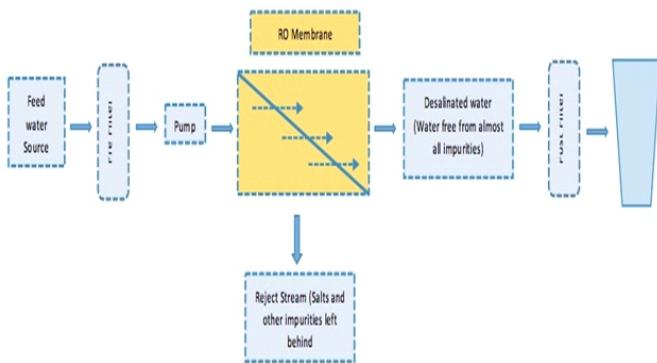
RO (Reverse Osmosis) is just the opposite of the above process. Osmosis occurs naturally without requirement of any energy from an external source. But in reverse osmosis (RO) pressure is applied to the higher concentrated solution. This energy or pressure should be higher than the natural osmotic pressure. Because of this pressure, salt water moves towards the fresh water through the semipermeable membrane, leaving behind the impurities, bacteria and ions.





Reverse Osmosis

Inside our RO (Reverse Osmosis) firstly the water from the supply or tap (called the feed water) enters the pre-filter where the filter cartridges remove the initial dirt and sand. Then with the help of a pump this filtered water (which still has lots of impurities and salts) is made to pass through the semi-permeable RO membrane. This semipermeable membrane allows only the water to pass through it leaving almost all the dissolved salt, impurities and bacteria behind called the reject stream. The amount of pressure required depends on the salt concentration of the feed water. Higher is the concentration of feed water, higher is the pressure required to overcome the osmotic pressure. The water coming out of the RO membrane is called Desalinated water. This water is free from almost all impurities. And finally this RO filtered water is passed through the post filters or the carbon filters which remove any remaining odour or taste from the water. Now your water is purified and ready to drink.



Functionality of RO water purifier

You need to get your RO service done in every three months, which includes cleaning or change of filters and membrane along with the cleaning of water storage tank.

RO technology is advisable in the areas where the quality of water is hard and high in TDS.

### UF purification

UF (Ultra filtration) purification method uses membrane similar to RO membranes but with bigger pores. UF membranes essentially removes all colloidal particles including most pathogenic organisms and turbidity but fails to remove the dissolved solids and salts. Unlike UV purifier, UF water purifier physically removes all the germs and bacteria from the water. Ultra-filtration water purifiers are as good as RO water purifiers when the water supplied to homes is not very hard and has less TDS. RO is only required when the water has too much TDS and hardness.

### Activated carbon filter purifier

This water purifier use activated carbon filters for purification process. The carbon filters can remove chemicals like chlorine, pesticides and impurities to a great extent. The filtration changes the taste and odour of water. It does not require electricity for operation. But it is also not very effective in removing microbes from water.

### Ion exchange



Resin beads used in ion exchange water treatment.

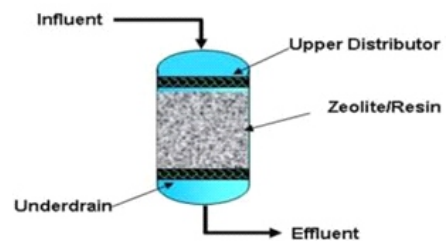
Ion exchange is a water treatment process commonly used for water softening or demineralization, but it is also used to remove other substances from the water in processes such as dealkalization, deionization, and disinfection.

### But what exactly is it?

Ion exchange describes a specific chemical process in which unwanted dissolved ions are exchanged for other ions with a similar charge.

Ions are atoms or molecules containing a total number of electrons that are not equal to the total number of protons.

There are two different groups of ions, cations, which are positively charged, and anions, which are negatively charged. We have Michael Faraday to thank for these names, which he devised based on the cation's attraction to the cathode and the anion's attraction to the anode in a galvanic device.



A typical ion exchange configuration.

### Removing Ionic Contaminants

This attraction is used to remove dissolved ionic contaminants from water. The exchange process occurs between a solid (resin or a zeolite) and a liquid (water). In the process, the less desired compounds are swapped for those that are considered more desirable. These desirable ions are loaded onto the resin material. In the exchange of cations during water treatment, positively charged ions that come into contact with the ion exchange resin are exchanged with positively charged ions available on the resin surface, usually sodium.

In the ion exchange process, negatively charged ions are exchanged with negatively charged ions on the resin surface, usually chloride. Various contaminants — including nitrate, fluoride, sulfate, and arsenic — can all be removed by anion exchange.

These resins can be used alone or in concert to remove ionic contaminants from the water.

If a substance is not ionic, such as benzene, it cannot be removed via ion exchange.

#### **Ion Exchange in Drinking Water Treatment**

Recently ion exchange resins have been increasingly used to create drinking water. Specialized resins have been designed to treat various contaminants of concern, including perchlorate and uranium.

There are many resins designed for these purposes, such as strong base/strong anion resin, which is used to remove nitrates and perchlorate. There are also resin beads that can be used for water softening.

#### **Recharging Resins**

Resin materials have a finite exchange capacity. Each of the individual exchange sites will become full with prolonged use. When unable to exchange ions any longer, the resin must be recharged or regenerated to restore it to its initial condition. The substances used for this can include sodium chloride, as well as hydrochloric acid, sulfuric acid, or sodium hydroxide.

The spent regenerant is the primary substance remaining from the process. It contains not only all of the ions removed, but also any extra regenerant ions and will also have a high level of total dissolved solids. It can be treated in a municipal waste water facility, but discharges may require monitoring.

The efficacy of ion exchange for water treatment can be limited by mineral scaling, surface clogging, and other issues that contribute to resin fouling.

Pre-treatment processes such as filtration or addition of chemicals can help reduce or prevent these issues.

#### **REFERENCES:**

<https://www.leaf.tv/articles/definition-of-pure-water/>  
<https://www.mrright.in/ideas/appliances/small-appliances/ro/how-does-reverse-osmosis-or-ro-water-purifier-works/>  
<https://www.mrright.in/ideas/appliances/small-appliances/ro/5-types-of-advanced-water-purification-technologies/>  
<https://www.fluencecorp.com/what-is-ion-exchange>  
[https://www.google.co.in/search?q=different+types+of+water+purification+methods&source=lnms&tbm=isch&sa=X&ved=0ahUKEwis1NST6\\_raAhUE2o8KHU6ICbIQ\\_AUICigB&biw=1280&bih=918#imgdii=c36MVG8NjE8daM:&imgcr=nhw8fHv8Wf6BYM](https://www.google.co.in/search?q=different+types+of+water+purification+methods&source=lnms&tbm=isch&sa=X&ved=0ahUKEwis1NST6_raAhUE2o8KHU6ICbIQ_AUICigB&biw=1280&bih=918#imgdii=c36MVG8NjE8daM:&imgcr=nhw8fHv8Wf6BYM)

## Paul Ehrlich

In 1906 Ehrlich prophesied the role of modern-day pharmaceutical research, predicting that chemists in their laboratories would soon be able to produce substances that would seek out specific disease-causing agents. He called these substances “magic bullets.” Ehrlich himself met with signal successes in the emerging fields of serum antitoxins and chemotherapy.



### Early Work with Dyes

Ehrlich was born near Breslau—then in Germany, but now known as Wrocław, Poland. He studied to become a medical doctor at the university there and in Strasbourg, Freiburg im Breisgau, and Leipzig. In Breslau he worked in the laboratory of his cousin Carl Weigert, a pathologist who pioneered the use of aniline dyes as biological stains. Ehrlich became interested in the selectivity of dyes for specific organs, tissues, and cells, and he continued his investigations at the Charité Hospital in Berlin. After he showed that dyes react specifically with various components of blood cells and the cells of other tissues, he began to test the dyes for therapeutic properties to determine whether they could kill off disease-causing microbes. He met with promising results using methylene blue to kill the malaria parasite.

### Antitoxins from Blood Sera

After a bout with tuberculosis and his subsequent cure with tuberculin therapy, developed by fellow German Robert Koch, Ehrlich focused his attention on bacterial toxins and antitoxins. At first he worked in a small private laboratory, but then he was invited to work at Koch's Institute for Infectious Diseases in Berlin. The post-Pasteur era was an exciting time to be looking for cures and preventives, and Koch's Institute was one of the best places to be. Among Ehrlich's new colleagues were Emil von Behring and Shibasaburo Kitasato, who had recently developed “serum therapies” for diphtheria and tetanus. Whereas Louis Pasteur's vaccines and Koch's tuberculin were made from weakened bacteria, these new serum therapies used blood serum, or cell-free blood liquid, extracted from the blood of naturally or artificially immunized animals to induce immunity. Von Behring and Kitasato evolved the concept of “antitoxin” to explain the immunizing properties of sera. One of Ehrlich's jobs at the institute was to make von Behring's diphtheria antitoxin in quantity and later to review the quality of the product produced by the chemical-pharmaceutical company Hoechst. In carrying out this work, he determined how to boost immunity systematically and how to produce high-grade sera.

In recognition of Ehrlich's accomplishments and of his promise as a researcher, in 1896 the Institute for Serum Research and Serum Testing was established for him in a Berlin suburb. In 1899 the institute moved to Frankfurt to more suitable quarters and was renamed the Royal Prussian Institute for Experimental Therapy.

### A Nobel Prize and Magic Bullets

In 1908 Ehrlich shared the Nobel Prize in Physiology or Medicine with Élie Metchnikoff for their separate paths to an understanding of the immune response: Ehrlich presented a chemical theory to

explain the formation of antitoxins, or antibodies, to fight the toxins released by the bacteria, while Metchnikoff studied the role of white blood corpuscles (phagocytes) in destroying bacteria themselves. By that time most scientists agreed that both explanations of the immune system were necessary.

Early in his career Ehrlich began to develop a chemical structure theory to explain the immune response. He saw toxins and antitoxins as chemical substances at a time when little was known about their exact nature. Up to that time, those scientists who were synthesizing therapeutic agents came at their tasks with few hypotheses about where and how these agents interacted with living systems. Ehrlich supposed that living cells have side chains—a shorter chain or group of atoms attached to a principal chain in a molecule—much in the way that dye molecules were known to have side chains that were related to their coloring properties. These side chains can link with particular toxins. According to Ehrlich, a cell under threat from foreign bodies grows more side chains, more than are necessary to lock in foreign bodies in its immediate vicinity. These “extra” side chains break off to become antibodies and circulate throughout the body. It was these antibodies, in search of toxins, that Ehrlich first described as magic bullets.

### Chemotherapy

Serum therapy was for Ehrlich the ideal method of contending with infectious diseases. In those cases, however, in which effective sera could not be discovered, Ehrlich would turn to synthesizing new chemicals, informed by his theory that the effectiveness of a therapeutic agent depended on its side chains. These “chemotherapies” were to be the new magic bullets.

In Frankfurt, Ehrlich turned from his work on serum therapy to chemotherapies and dyes. First targeting the protozoa that were known to be responsible for certain diseases, such as sleeping sickness, he and the Japanese bacteriologist Kiyoshi Shiga synthesized trypan red as a highly effective cure for that disease. In 1906 Georg-Speyer-Haus, a research institute for chemotherapy, was established with its own staff under Ehrlich's direction. Soon this institute and the Hoechst and Cassella chemical companies reached an agreement that gave the companies the right to patent, manufacture, and market preparations discovered by Ehrlich and his colleagues. The companies further agreed to supply chemical intermediates for the syntheses that the staff of the institute would undertake.

### Salvarsan

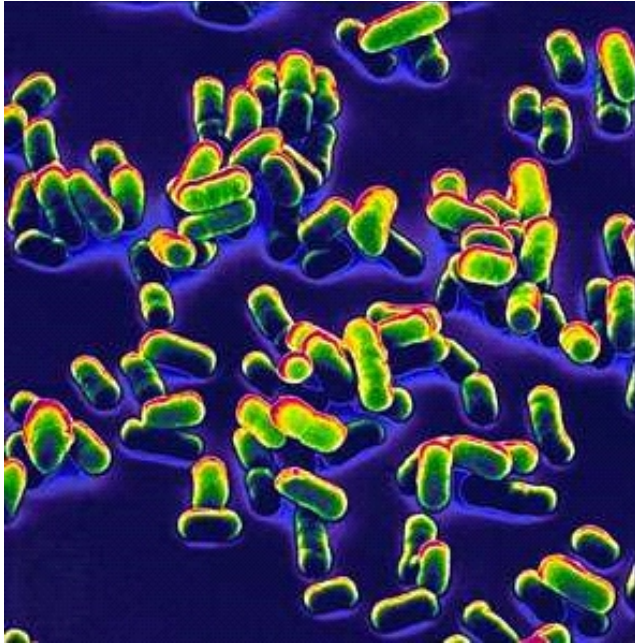
The researchers, now including an organic chemist, Alfred Berthel, and a bacteriologist, Sahashiro Hata, broadened the targeted microorganisms to include spirochetes, which had recently been identified as the cause of syphilis. Beginning with an arsenic compound, atoxyl, in three years' time and three hundred syntheses later—for that day an amazingly large number—they discovered Salvarsan (1909). Salvarsan was first tried on rabbits that had been infected with syphilis and then on patients with the dementia associated with the final stages of the disease. Astonishingly, several of these “terminal” patients recovered after treatment. More testing revealed that Salvarsan was actually more successful if administered during the early stages of the disease. Salvarsan and Neosalvarsan (1912) retained their role as the most effective drugs for treating syphilis until the advent of antibiotics in the 1940s.

## Multiple Choice Quiz

- 1) **The pore size of 'nitrocellulose' is**  
 A. 0.23  $\mu\text{m}$   
 B. 0.22  $\mu\text{m}$   
 C. 0.21  $\mu\text{m}$   
 D. 0.26  $\mu\text{m}$
- 2) **The temperature range for 'pasteurization' is**  
 A. 60°C-70°C  
 B. 62°C-72°C  
 C. 65°C-75°C  
 D. 121°C-130°C
- 3) **Removal and killing of all microorganisms is known as**  
 A. Destruction  
 B. Sterilization  
 C. Pasteurization  
 D. Removal
- 4) **The 'filters' that are commonly used, known as**  
 A. Nitrocellulose  
 B. Filtration tubes  
 C. Sieves  
 D. Filter paper
- 5) **Sterilization is done by autoclave, consisting exposure to steam, about**  
 A. 120°C  
 B. 170°C  
 C. 121°C  
 D. 116°C
- 6) **The time duration for the 'pasteurization' is**  
 A. 30 minutes  
 B. 20 minutes  
 C. 15 minutes  
 D. 11 minutes
- 7) **During 'pasteurization' the milk is heated for 30 minutes at**  
 A. 67°C  
 B. 63°C  
 C. 62°C  
 D. 61°C
- 8) **In hospitals, the surgical instruments and plastics are washed with**  
 A. Ethylene oxide  
 B. Iodine  
 C. Tincture  
 D. Chlorine
- 9) **For the purification of swimming pools and water supplies of chemical used is**  
 A. Alcohol  
 B. Chlorine  
 C. Iodine  
 D. Heavy metals
- 10) **Before the 'immunization' the skin is cleaned with**  
 A. Detergents  
 B. Insecticides  
 C. Alcohol  
 D. Ethanol
- 11) **The method used for the sterilization of solutions is called**  
 A. Sterilization  
 B. Autoclaving  
 C. Filtration  
 D. Radiations
- 12) **Contact lenses and wounds are cleaned by an antiseptic, named as**  
 A. Iodine  
 B. Tincture  
 C. Hydrogen peroxide  
 D. Chlorine

Answers: 1) B 2) B 3) B 4) A 5) C 6) A 7) D 8) A 9) B 10) D 11) C 12) C

## Yersinia



Yersiniosis is a relatively uncommon infection contracted through the consumption of undercooked meat products (especially pork), unpasteurized milk, or contaminated water. Usually, someone with an infection caused by Yersinia bacteria recovers within a few days without medical treatment (in some cases, doctors prescribe antibiotics).

### About Yersiniosis

Of the three main types of yersiniosis that affect people, Yersinia enterocolitica (bacteria that thrive in cooler temperatures) are responsible for most infections in the United States. The infection seems to be more common in cooler climates.

The bacteria can infect the digestive tracts of humans, cats, dogs, pigs, cattle, and goats. People can contract it by eating or handling contaminated foods (such as raw or undercooked meat) or by drinking untreated water or unpasteurized milk that contain the bacteria.

An infant can be infected if a parent or caretaker handles contaminated food without cleaning up adequately before handling the baby's toys, bottles, or pacifiers.

### Signs and Symptoms

Symptoms of yersiniosis appear 4–7 days after exposure and can last up to 3 weeks. They include fever, stomach pain, nausea, vomiting, and bloody diarrhea. Sometimes, older kids also get pain in the lower right side of the abdomen, which can mimic appendicitis. Some people also have a sore throat along with other symptoms.

If your child has these symptoms, call your doctor. For infants, it's particularly important to call the doctor as soon as symptoms appear to prevent the infection from leading to other health problems.

In rare cases, the infection can cause a skin rash called erythema nodosum, or joint pain that appears a month after the initial

symptoms. The rash usually appears on the legs and trunk. The joint pain is usually in the larger joints and is thought to be due to an immune system response. These symptoms usually go away with time but can last several months.

The diagnosis of Yersinia can be confirmed with a stool culture. If the Yersinia infection leads to an infection of the blood, known as bacteremia, it can be confirmed with a blood culture.

### Treatment

Diarrhea caused by yersiniosis generally goes away on its own, though in some cases antibiotics are prescribed. In infants, however — particularly those who are 3 months old or younger — it can develop into bacteremia. Infants who contract yersiniosis are usually treated in a hospital.

Depending on the severity of the diarrhea, your doctor may suggest modifying your child's diet for 1 or 2 days and encouraging your child to drink more fluids (which may include drinks with electrolytes to replace body fluids quickly).

If your child has frequent bouts of diarrhea, watch for signs of dehydration, including:

- severe thirst
- dry mouth or tongue
- sunken eyes
- dry skin
- not peeing as often as usual
- in infants, a dry diaper for several hours
- no tears when crying
- looking lethargic

### Prevention

To reduce the risk of yersiniosis, take these precautions:

- Don't serve or eat raw or undercooked meat.
- Drink and serve only pasteurized milk and milk products.
- Wash hands with soap and water particularly before eating and preparing food; before touching infants or their toys, bottles, or pacifiers; and after contact with animals or handling raw meat.
- Use separate cutting boards for meat and other foods.
- Clean all cutting boards, countertops, and utensils with soap and hot water after preparing raw meat.
- Always cook meat thoroughly before you eat it, especially pork products.
- Dispose of animal feces and sanitize anything they have touched.
- Avoid drinking directly from natural water sources such as ponds and mountain streams, particularly if the water is near farmland where cattle, pigs, or goats are raised.
- As you care for a family member who has diarrhea, remember to wash your hands thoroughly before touching other people and before handling food.
- If your pet dog or cat has diarrhea, wash your hands frequently as you care for it, and check with your veterinarian about treatment and/or contagiousness.

# What is Q Fever

Q fever, also called query fever, is a bacterial infection caused by the bacteria *Coxiella burnetii*. The bacteria are most commonly found in cattle, sheep, and goats around the world. Humans typically get Q fever when they breathe in dust that was contaminated by infected animals. Farmers, veterinarians, and people who work with these animals in labs are at the highest risk of being infected. The highest amounts of bacteria are found in the "birth products" (placenta, amniotic fluid) of infected animals.

The disease may cause mild symptoms similar to the flu. However, many people have no symptoms at all. Mild forms of the disease may clear up in a few weeks without any treatment.

In rare cases, a more serious form of disease develops if the infection is chronic, which means it persists for six months (and there are some case reports indicating that it may persist for more than six months). A more serious form also can develop if the infection is recurrent, which means it comes back. People with heart valve problems or weak immune systems are at the highest risk of developing these types of Q fever. Chronic Q fever is very serious because it can damage a person's vital organs, including the:

- heart
- liver
- brain
- lungs

More severe or chronic forms of Q fever can be treated with antibiotics. Those at risk for Q fever can prevent the disease by disinfecting contaminated areas and washing their hands thoroughly.

## What Are the Symptoms of Q Fever?

Symptoms of Q fever don't typically appear until about two to three weeks after exposure to the bacteria. However, it's possible that you will have the infection and not show any symptoms. If symptoms do appear, they're generally mild.

Symptoms can vary significantly from one person to another. Common symptoms of mild Q fever may include:

- a high fever
- chills or sweats
- a cough
- chest pain while breathing
- a headache
- clay-colored stools
- diarrhea
- nausea
- abdominal pain
- jaundice
- muscle pain
- shortness of breath

A rash is also a symptom, but it's not common.

## What Causes Q Fever?

Q fever is caused by a bacterial infection with a bacterium called *Coxiella burnetii*. The bacteria are typically found in cattle, sheep, and goats. The animals transmit the bacteria in:

- urine
- feces
- milk

- fluids from giving birth

These substances can dry inside a barnyard where contaminated dust can float in the air. Humans get Q fever when they breathe in the contaminated air.

In rare cases, drinking unpasteurized milk can cause infection. The bacteria cannot be spread directly from one human to another. The exact frequency of Q fever isn't known because most cases aren't reported.

## Who Is at Risk for Q Fever?

Since the bacteria usually infect cattle, sheep, and goats, people who are at highest risk for infection include:

- farmers
- veterinarians
- people who work around sheep
- people who work in the dairy industry
- people who work in a meat processing facilities
- people who work in research laboratories with livestock
- people who work in research laboratories with *C. burnetii*
- people who live close to a farm

## How Is Q Fever Diagnosed?

It's difficult for a doctor to diagnose Q fever based on symptoms alone.

Your doctor may suspect you have Q fever if you work or live in an environment that puts you at high risk for exposure and you have any of the flu-like symptoms or serious complications of Q fever. Your doctor might ask you questions about your job or if you've recently been exposed to barnyard or farm animals.

Q fever is diagnosed with a blood antibody test. According to the Centers for Disease Control (CDC), an antibody test frequently appears negative in the first seven to 10 days of sickness. Your doctor should use their best judgment to decide whether or not to begin treatment based on suspicion alone.

If your doctor suspects you have a chronic infection, they may order a chest X-ray and other tests to look at your lungs and a test called an echocardiogram to look at your heart valves.

## What Are the Complications of Q Fever?

Sometimes Q fever can persist or come back. This can lead to more serious complications if the infection affects your:

- heart
- liver
- lungs
- brain

You're at high risk of developing chronic Q fever if you:

- have an existing heart valve disease
- have blood vessel abnormalities
- have a weakened immune system
- are pregnant

According to the CDC, chronic Q fever occurs in less than 5 percent of infected patients. The most common and serious complication of Q fever is a heart condition called bacterial endocarditis. Endocarditis is the inflammation of the inside lining of the heart chambers and heart valves, which is called the endocardium. This can cause damage to your heart valves and may be fatal if it isn't treated.

Other serious complications are less common. They include:

- pneumonia or other lung issues
- pregnancy problems, such as miscarriage, low birth weight, premature birth, and stillbirth
- hepatitis, which is an inflammation of the liver
- meningitis, which is an inflammation of the membrane around your brain or spinal cord

#### **How Is Q Fever Treated?**

Treatment depends on the severity of symptoms.

##### **Mild Infection**

The milder forms of Q fever usually resolve within a few weeks without any treatment at all.

##### **More Severe Infection**

Your doctor will prescribe an antibiotic. Doxycycline is the antibiotic of choice for all adults and children with severe Q fever. You should begin taking it immediately if Q fever is suspected to be the cause of your illness, even before laboratory results are available.

Standard duration of treatment is two to three weeks. The symptoms, including fever, should subside within 72 hours. Failure to respond to doxycycline may suggest that the illness isn't Q fever.

##### **Chronic Infection**

Antibiotics are typically given for 18 months if you have chronic Q fever.

#### **What Is the Outlook After Treatment?**

Antibiotics are usually very effective, and fatality from the disease is very uncommon. People with endocarditis, however, need an early diagnosis and antibiotics for at least 18 months for a successful outcome.

#### **How Can Q Fever Be Prevented?**

A vaccine has been successful in Australia for people who work in high-risk environments, but it isn't currently available in the United States.

If you're at high risk for Q fever and you aren't vaccinated, you should take the following preventive steps:

- Properly disinfect and decontaminate exposed areas.
- Properly dispose of all birth materials after a livestock animal has given birth.
- Wash your hands properly.
- Quarantine infected animals.
- Make sure the milk you drink is pasteurized.
- Test animals routinely for infection.
- Restrict the airflow from barnyards and animal holding facilities to other areas.

## Diabetic Foot Treatment (part 2)



Foot pain is one of the adverse side effects of diabetes. For this reason, foot care is integral to diabetic care and management. Unattended feet can become problematic and painful, sometimes, leading to amputation as well. Depending on the **extent of pain and damage**, medications can be given although the first line of action is to control the blood glucose level.

### Pain from Diabetic Peripheral Neuropathy

An estimated 60-70 percent of people with diabetes have some form of neuropathy, making it one of the most common complications of diabetes. The symptoms of diabetic peripheral neuropathy (DPN)—nerve damage in the extremities—are most often felt in the toes, feet, and hands.

Common descriptions of the feelings or sensations for DPN are:

- Burning
- Numbness
- Tingling
- Stinging
- Electrical vibrations
- Shooting pain
- Searing pain

### CONTROL OF BLOOD GLUCOSE LEVEL

The primary treatment for **diabetic foot pain** is to **bring blood glucose levels within the normal range**. This is essential to hinder further damage to the nerves and related parts. Although blood glucose control can worsen symptoms initially, over time, this helps in reducing the symptoms. Problems with the feet may also force you to consult a foot care specialist depending on the damage.

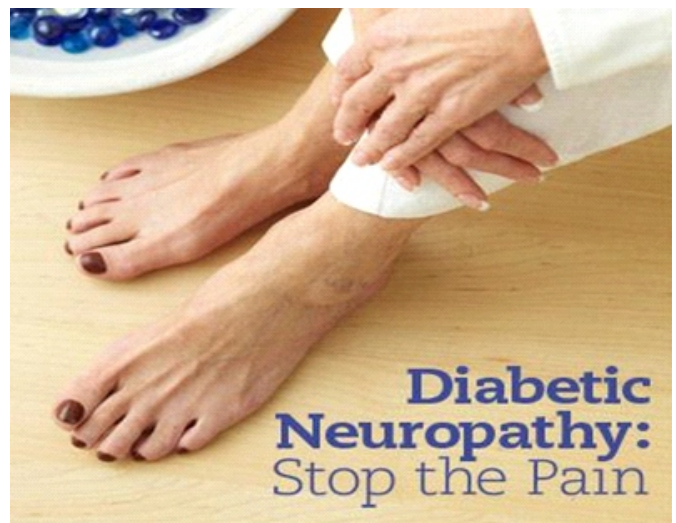
### MEDICAL TREATMENT FOR FOOT PAIN

Medication can be administered for **wounded or infected feet** or if the doctor suspects risk of infection. If antibiotics are prescribed, take the entire course even though positive results can appear within two to three days only. For treating painful **diabetic neuropathy**, Cymbalta and Lyrica are the FDA-approved medications. In case of a severe infection, the doctor can advise hospitalization as giving pills may seem ineffective.

There can be a single dose of antibiotics as a **shot or IV dose** before starting pills. Diabetic foot treatment can also be done at

several wound care centers which focus on the lower extremity wounds and ulcers. Debridement of the wound through surgery, improvement of circulation and special dressings can be some of the other treatment procedures.

Depending on the need, a **combination of treatments** can also be implemented. In some cases where there is a bone-related problem, flat feet, arthritis, etc. you may need to consult a specialist as an orthopedic surgeon. Besides taking care of your existing problem, the specialist can also provide an excellent resource for how to care for your feet on daily basis. Diabetic foot pain treatment may also be dealt with shoe inserts, removal of calluses, etc.



### CARE THROUGH LIFESTYLE CHANGES

There are many things which aid **diabetes foot pain treatment** and delay worsening of the damage. Routine examination of the feet helps identify any damage even though it is trivial. In case of any injury, **attend to it immediately as even a small injury** can create havoc for the foot. Use cotton or woolen socks as elastic socks (and even hosiery) can hinder circulation. Besides, choose comfortable shoes to protect the feet. If you are unsure of the fitness of your footwear, consult a podiatrist.

Regular exercising can **keep bones and joints in good health**. It is good to lower blood sugar and consequently manage diabetes complications like foot pain. High impact exercises may not suit all, particularly when you have lost sensation in the feet. Consult the doctor when adopting an exercise program.

Keep nails trimmed to avoid damaging them. Apply a water-based moisturizer regularly to prevent cracking but avoid using it between your toes. Smoking adversely affects blood vessels by increasing the damage already caused.

### SELF CARE THROUGH OTHER THINGS

Diabetic foot pain can be treated through other ways depending on the symptoms. Self care through warm baths can relieve you of the pain. Frequent walking can also soothe peripheral nerve pain.



If you do not have cuts or wounds, bathing and walking may be helpful in reducing foot pain.

A suited diet can also take care of foot (and nerve) pain by emphasizing on blood sugar control. Try to keep track of what you eat, when and how much you eat. Remembering everything can be cumbersome, so concentrate on the major factors contributing to good glucose control.

Sometimes, creams and over-the-counter pain suppressers can help. **Lidocaine or capsaicin** containing creams may prove useful in controlling pain to certain extent. As far as over-the-counter medications are concerned, ibuprofen, naproxen and aspirin may be useful. But remember that over-the-counter pain medicines may not work well can pose (serious) side effects as well.

**USING ANTIDEPRESSANTS TO RELIEVE PAIN**

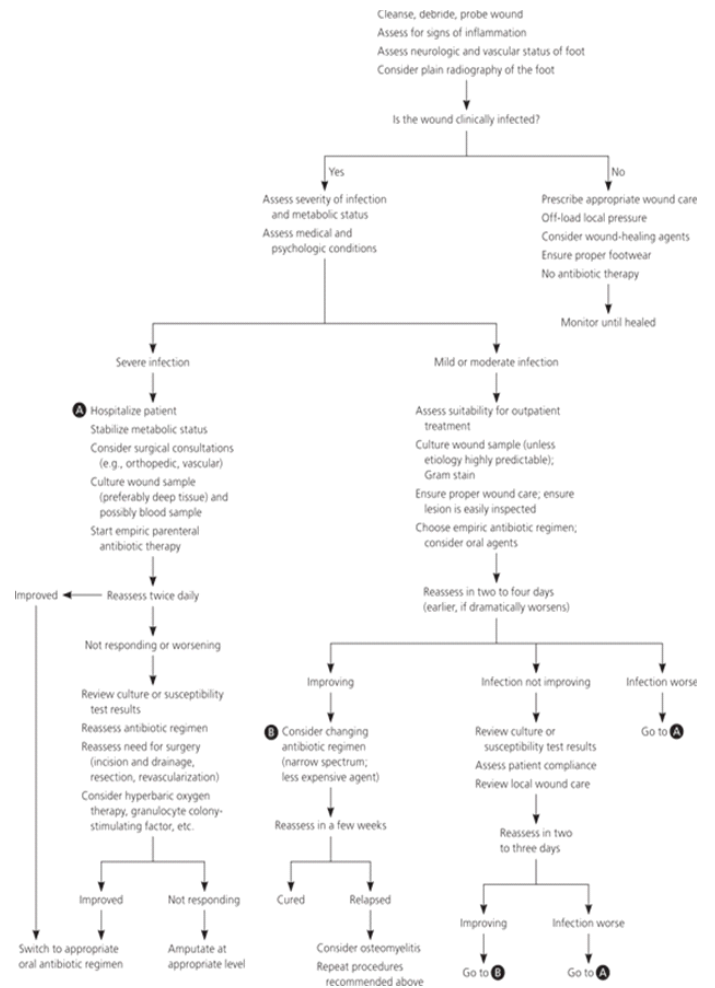
Antidepressants may also relieve diabetic nerve pain. Even if there is absence of clinical depression in a patient, doctors may prescribe antidepressants to cater to foot pain and other nerve pain.

Antidepressants have the ability to deal with both pain and depression and can be used as a part of diabetic foot pain treatment.

**SWITCHING THERAPIES FOR BETTER CONTROL**

Changing a therapy can, sometimes, be a **helpful technique in combating foot and nerve pain**. A study pointed out that insulin injections used for glucose control can reduce the likelihood of neuropathy.

Therapies like acupuncture, transcutaneous electrical nerve stimulation (TENS), anodyne therapy can also **relieve diabetic foot pain**. Another technique which can be helpful in diabetes foot pain treatment is the Dellon procedure. It is a surgical decompression of multiple peripheral nerves. Administering vitamin B12 can also be helpful, although it has its limitations.



**REFERENCES:**

[https://www.google.co.in/search?q=diabetic+foot+treatment+soothing+pictures&source=lnms&tbn=isch&sa=X&ved=0ahUK E w j R 0 6 q x u v X a A h V B u Y 8 K H Y E b A - w Q \\_ A U I C i g B &biw=1280&bih=918#imgrc=miYJ8o6BQnJeJM](https://www.google.co.in/search?q=diabetic+foot+treatment+soothing+pictures&source=lnms&tbn=isch&sa=X&ved=0ahUK E w j R 0 6 q x u v X a A h V B u Y 8 K H Y E b A - w Q _ A U I C i g B &biw=1280&bih=918#imgrc=miYJ8o6BQnJeJM)  
<http://www.findhealthremedies.com/6-effective-treatment-of-diabetic-foot-pain/>  
<http://www.diabeticlivingonline.com/complications/feet/stop-pain-diabetes-nerve-damage>

# Microexpress®

Introduces

## MUCROPRO™-AST

Antimicrobial Susceptibility Testing

MUCROPRO™-AST is a system Intended for Antimicrobial Susceptibility Testing of most pathogens involved in UTI, GI, GT, ENT, CNS, Blood etc. Results can be delivered within 5-8 hours.

- ✓ Spectrophotometric Turbidimetric Technology.
- ✓ 91.67% Correlation with Standard Kirby Bauer Method.
- ✓ Applicable to all pathogens from any type of Infection.
- ✓ Facilitates AST results within 24 hours of receiving the sample.
- ✓ Optimizes Lab Work Easy sample preparation.
- ✓ Automated result interpretation Simple Procedure Adaptable by almost all Laboratories.



### Installation Pack

MUCROPRO™-AST  
Analyzer with accessories

MUCROPRO™-AST  
Multichannel Micropipette

Mcfarland Std. 0.5

### Reagent Pack

MUCROPRO™-AST  
Susceptibility Test Panel Kit-UTI

MUCROPRO™-AST  
Susceptibility Test Panel Kit-GN

MUCROPRO™-AST  
Susceptibility Test Panel Kit-GP

### Accessory Pack

MUCROPRO™-AST  
Gamma Sterile Tips

MUCROPRO™-AST  
Test Panel Tray with Tray cover

Gamma Sterile Loop, Dropper  
and Reservoir

BioShields® Presents

### NUSEPT™

Wound healing with perfect balance between Antisepsis & re-epithelization.

NUSEPT™ is a clear, green coloured, new generation, powerful, microbicidal antiseptic solution. It is safe and highly effective for medical, surgical and general purpose antiseptics.

COMPOSITION : ● 1%v/v Poly (hexamethylene biguanide) hydrochloride (PHMB) ● Perfume ● Fast green FCF as colour

CONTACT TIME : ● 1 minute (undiluted & 10% v/v solution)

● 5 minutes (5% v/v solution) ● 10 minutes (2.5% v/v solution)

ACTIVITY : Broad spectrum : Bactericidal, Fungicidal and Virucidal



| Features                         | Benefits   |
|----------------------------------|--|
| Structurally similar to AMPs**   | Enhance the immune response by functioning as immunomodulators |
| Maintain hydrobalance            | Facilitate wound healing                                       |
| Anti-biofilm effect              | Effective in chronic & diabetic wounds                         |
| BI***>1                          | ● Non cytotoxic<br>● Helps in re-epithelization                |
| No known resistance              | Effect against wide range of microbes                          |
| ● Non-stinging<br>● Non-staining | Good patient compliance  |

### APPLICATIONS :

- Pre & post surgery skin and mucous membrane antisepsis
- Surgical and non-surgical wound dressings
- Chronic wound (Diabetic foot ulcers, pressure ulcers, arterial/venous leg ulcers) management
- Routine antisepsis during minor incisions, catheterisation, scopy etc
- First aid

### USAGE DIRECTIONS :

- Pre & post-surgery skin cleaning & antisepsis : Use undiluted
- Surgical, post operative, non surgical dressing : Use undiluted, once day/alternate
- Antisepsis during minor incisions, scopy, catheterization, first aid, cuts, bites, stings etc : Use undiluted
- Chronic wound management (diabetic foot, pressure and arterial/venous leg ulcers) : Use undiluted
- First aid : Use undiluted

\*\*AMPs- Antimicrobial Peptides

\*\*\*BI-Biocompatibility Index measures an antiseptic agent's antimicrobial activity in relation to its cytotoxicity

Not recommended for infants below 9 months except on medical advice.

## Highlights of the coming issue

