ENZYMATIC DETERGENTS

EmPower, MetriZyme, DetergeZyme

Technical Bulletin

The importance of cleaning instruments prior to disinfection

Reusable medical instruments are required to be properly cleaned and disinfected between each use. Cleaning is defined as the removal of all foreign material such as blood, protein, cellular debris, tissue, respiratory secretions, mucus, saliva, feces, etc. from objects. If the instrument is not clean, the organic soil can harbor embedded microorganisms. Organic soil can prevent the penetration of the germicide and render the disinfection or sterilization process ineffective. Additionally, some disinfectants such as hypochlorites are inactivated by organic material.[i]

The definition and properties of enzymes

All enzymes are proteins but not all proteins are enzymes. Enzymes are distinguished from other proteins by their ability to catalyze certain chemical reactions. A catalyst is defined as a substance that accelerates a chemical reaction without being consumed in the overall reaction. In detergents, enzymes known as proteases can break up proteins from blood and leftover tissues into component units (termed amino acids) or short chains of amino acids (termed peptides). Amino acids and peptides are more water soluble than intact proteins, therefore they are *much* easier to remove.

The benefits associated with incorporation of enzymes into detergents

Enzymes enhance detergent cleaning by breaking down large, hard to remove materials into smaller, easy to remove fragments. There are three basic types of enzymes used in detergents: proteases, amylases, and lipases. Proteases are the most important type of enzyme to look for when choosing an enzymatic detergent for medical use because there is a high content of protein in most body fluids (including blood, tissue and mucous) which cannot be easily removed with regular detergents/surfactants and water. Proteases break down protein into individual amino acids or short strings of amino acids (peptides). Amino acids and peptides are much more soluble in water and will float away from the surface of the instrument. Amylases are not essential components in enzymatic detergents because they have limited action on carbohydrates/starches. In addition, carbohydrates/starches are very soluble in water and tend to be easy to remove with most detergents/surfactants and water. Lipases in concert with bile are useful for removing lipids/fats in the body (in vivo), however, outside the body (in vitro) lipases have limited effectiveness. Lipases are soluble in water and lipids are insoluble in water. The lipase has to mix with the lipid in order to break it up via hydrolysis. Since hydrolysis only occurs at the interface between the lipid droplet and the aqueous phase (water), this reaction is relatively slow and ineffective. Appropriate detergents/surfactants are more effective at removing lipids from medical instruments than lipases.

The incorporation of enzymes into a detergent for endoscope cleaning has several benefits in addition to enhancing overall cleaning performance. Clogging of endoscope channels is virtually eliminated with appropriate enzymatic detergents. This reduces the need for costly routine maintenance and results in large savings. In addition, the film which tends to build up on the optical parts of an endoscope after successive use is efficiently removed. As a result, the quality of the images is improved. Properly

formulated enzymatic detergents, such as Metrex enzymatics, are non-corrosive and do not attack any metal surfaces on medical instruments. Properly formulated enzmatics will efficiently work in mild conditions and will not damage valves, rubber gaskets or any surface of a flexible fiberoptic endoscope or other medical instrument. Enzymes should also be fully biodegradable.

The properties and benefits of surfactants in enzymatic detergents
Surfactants are surface active agents with wetting, detergent and emulsifying properties. Surfactants have both hydrophilic (water loving) and hydrophobic (water avoiding) properties and play a key role in soil removal. Since hydrophobic regions on a medical instrument surface can prevent a disinfectant from contacting and disinfecting the contaminated surface, it is important to add a carefully selected surfactant as a wetting agent to the solution. Another benefit of a good surfactant in an enzymatic detergent is that it will prevent protein fragments from redepositing on a medical instrument.

Surfactants with good wetting properties will facilitate increased enzymatic action in an enzymatic detergent. Good surfactants can even replace lipase enzymes, which are far less efficient than surfactants in solubilizing and removing lipid/fat soils. It is for this reason that soaps and detergent components such as surfactants, rather than lipases, have been conventionally used for hand cleaners and laundry detergents.

Practical usage of enzymatic detergents
Enzymatic detergents are sold in concentrated form. Typically they are mixed 1-2 ounces concentrate per gallon of warm water prior to use in ultrasonic or manual cleaning systems. Enzymatic solutions are also appropriate for automated endoscope reprocessors and washer sterilizers/decontaminators.

It is important to change the diluted enzymatic detergent daily. In general, the concentrated form is bacteriostatic (not favorable to bacterial growth). Once the product is diluted, the solutions are generally not bacteriostatic so it is possible for bacteria to grow. In addition, the concentrated solution contains stabilizers for the enzymes. When the product is diluted, the enzymes become less stable and will denature over time. In layman's terms, this means the diluted product will develop an unpleasant smell in 2-4 days and can become a breeding ground for bacteria.

Metrex enzymatic detergents
Metrex produces three enzymatic detergents: MetriZyme, emPower and DetergeZyme. MetriZyme is a dual enzyme formula designed for superior performance in the presence of high organic debris and in areas that are hard to reach mechanically (such as endoscope channels). EmPower is formulated with a lower concentration of the same two high quality enzymes found in MetriZyme. Empower is an economical alternative to MetriZyme when there is moderate to low organic debris. DetergeZyme is a single enzyme formulation. DetergeZyme is a good choice for instrument cleaning and de-scaling in situations with minimal organic debris. Specific technical information on each product is detailed in the following paragraphs and charts.

MetriZyme is designed for cleaning reusable medical instruments and devices (such as endoscopes and surgical instruments) which may be soiled with protein, carbohydrate and lipid containing debris and which require subsequent disinfection or sterilization. MetriZyme is a concentrated cleaning solution comprising two protease enzymes (Alcalase 2.5L and Savinase 16.0L) and nonionic surfactants cleaning agents. Alcalase

and Savinase are serine-type subtilisin proteases with the broadest specificity of any commercially available enzymes for medical instrument cleaning. The combination of these two enzymes results in a cleaning solution with the broadest action on a variety of protein soils. The two nonionic surfactants provide significant additional cleaning action on carbohydrate and lipid soils as well as on protein soils. MetriZyme also contains propylene glycol (an enzyme stabilizer), a buffer to maintain pH, a preservative, an antifoaming agent, peppermint oil (for fragrance) and FD&C Blue Dye #1 (to differentiate it from water and other solutions).

EmPower has all the features and benefits of MetriZyme except the total enzyme concentration is 4%. This allows us to offer emPower at a lower price than MetriZyme. Additionally, emPower has powerful cleaning agents that will not get cloudy at the use temperature. Also, emPower is specifically designed to be low foaming for use in automated washers. EmPower is also specially formulated to be particularly non-corrosive to metals.

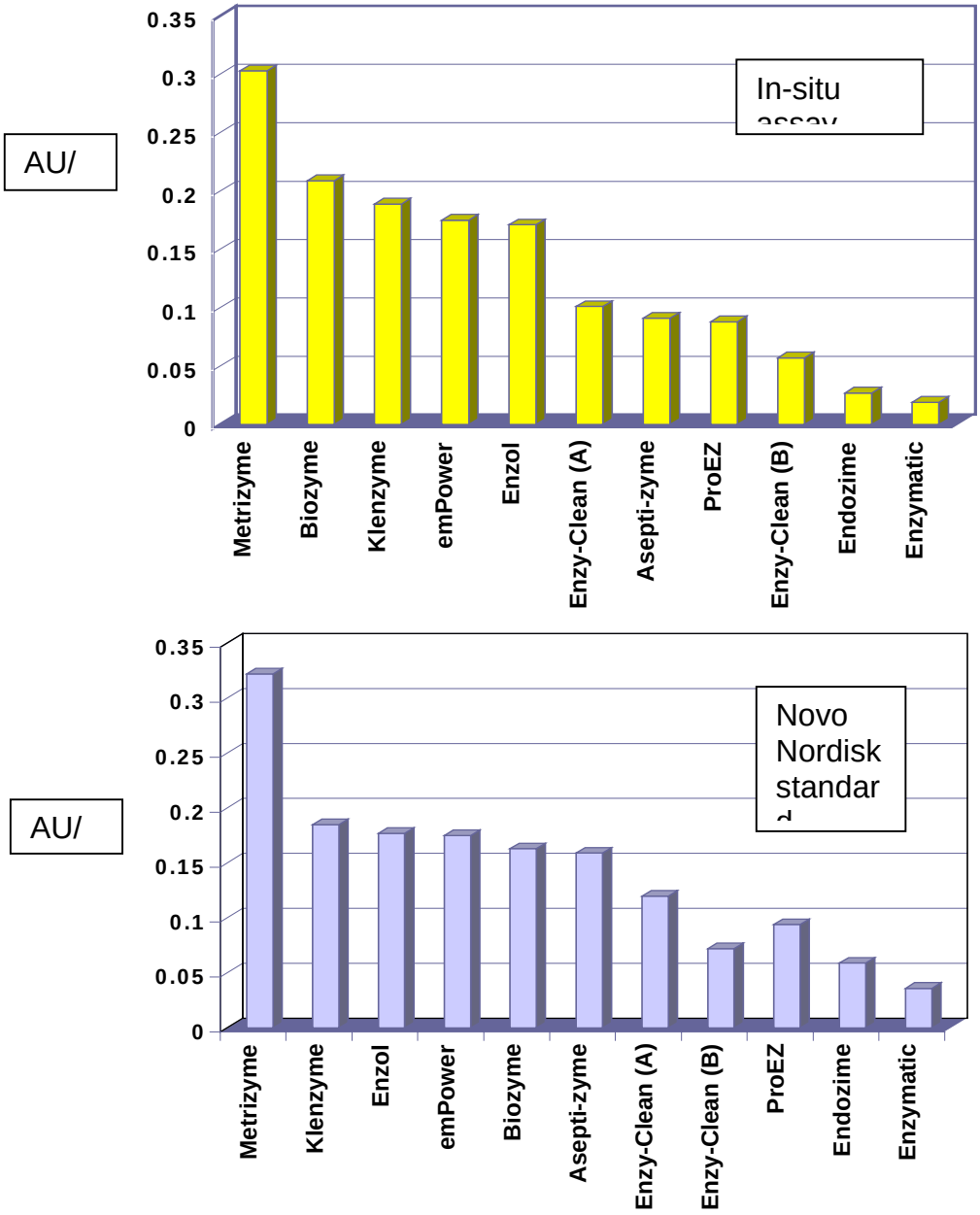
The DetergeZyme formulation contains a single enzyme (Savinase) with a total enzyme concentration of 5%. DetergeZyme also contains high quality surfactants and biodegradable detergents.

Metrex enzyme detergents should be diluted with at least one ounce of concentrate per gallon of warm water prior to use in manual, ultrasonic or automatic cleaning systems. Currently, the products are packaged in various sizes and configurations: a 55 gallon, 30 gallon, 15 gallon, 5 gallon, 1 gallon container with pump, 1/2 gallon container with pump, 1 quart container, 8 ounce bottle. All containers are blow molded HDPE. Caps for containers up to one gallon are polypropylene with induction welded inner seal. All one gallon or smaller containers are placed in cardboard cases.

Comparative assessment of Metrex and competitor enzyme products

Commercially available enzyme-based medical instrument pre-cleaners were tested for proteolytic enzyme activity, pH and foaming capacity to comparatively assess product performance. Table 1 presents results of proteolytic enzyme activity assays of diluted products, one ounce to one gallon of tap water, using two industry standard methods. Both methods utilize casein, the principle protein of bovine milk, as a standard substrate protein. The pH of the solution of enzyme and casein is not adjusted in the in-situ assay method. The pH of the enzyme plus casein solution is adjusted to 8.5 in the Novo Nordisk standard proteolytic enzyme activity assay. Novo Nordisk A/S is considered to be the top enzyme raw material producer in the world and is the supplier of Metrex enzyme raw materials.

TABLE 1 Proteolytic Enzyme Activity



The results presented in TABLE 1 show that MetriZyme has significantly more proteolytic enzyme activity than other commercially available products, when tested with either method. The results show that emPower has the second highest enzyme activity within statistical significance, equal to Biozyme, Klenzyme ® and Enzol ®. The results also show more than a ten-fold range in activity between the products tested.

TABLE 2 Summary of Product Label Information

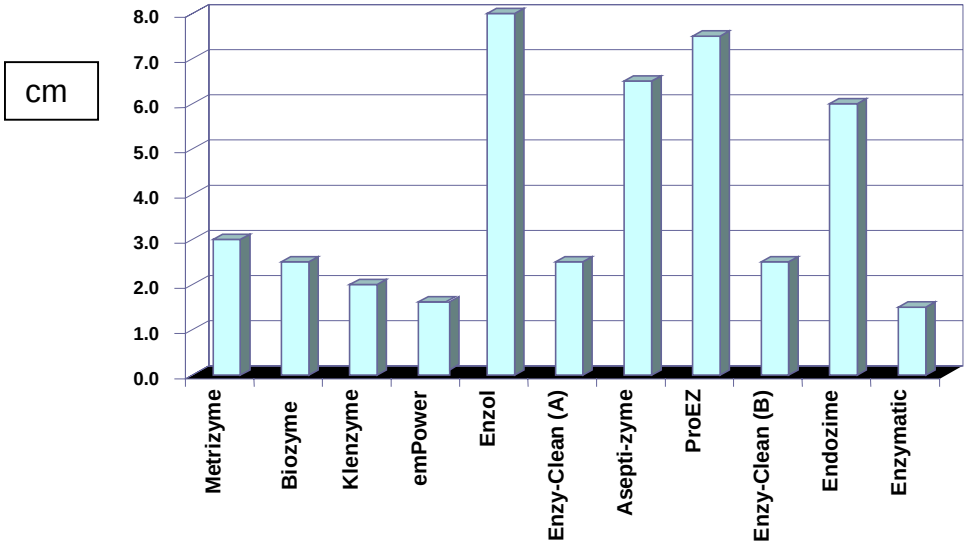
	Names	Mnfrs	Ingredients	Excipients	Label Statements
1	Metrizyme	Metrex	Proteolytic Enzymes12%, Nonionic Surfactants15%	*See data below	One step;low foaming;safe for users
2	Biozyme ID132	Biotrol	Subtilisins, Proteolytic Enzymes	xxxx	xxxx
3	Klenzyme®	Steris Corp.	Subtilisins, Proteolytic Enzymes #9014-01-0	xxxx	Enzymatic presoak
4	emPower™	Metrex	Proteolytic Enzymes, Nonionic Surfactants	*See data below	Non-corrosive; low foaming; safe
5	Enzol®	J&J	Proteolytic Enzymes	xxxx	One step;reduces exposure;reduces likelihood of corrosion to equipment
6	Enzy-Clean®	Alliance	Protease #9014-01-0, Amylase #9000-85-5	Propylene glycol, water	Rust inhibiting;biodegradable; PO4free;chloride free; digests fat and other oils
7	Asepti-zyme®	Huntington	Proteolytic Enzymes	Propylene gly., water	Low foam;non-staining
8	ProEz™	Cottrell	Protease, Amylase, Citric Acid	xxxx	Odor suppressant;fast;safe; gentle; economic-concentrated
9	Enzy-Clean	Baxter	Enzymatic Solution	xxxx	Biodegradable;PO4 free;chloride free rust inhibiting
10	Endozime	Ruhof	Protease, Amylase, Digesters, and Buffers	10% Isopropyl Alcohol	Free rinsing, bacteriostatic; cleans in 2-3 min;digest and dissolve fecal, urine,and vomital matter;will not harm anodized Al,plastics, or tubing
11	Enzymatic™	Sklar	Fermented Enzymes, Surfactants	xxxx	Cleans quickly;safe

*Propylene glycol, water, inerts

All of the products above have many features and benefits that they each claim on their labels which are common to all of the products. They all claim to be enzymatic detergents that can be used to clean medical instrumentation. Other common claims from the products are as follows: safe due to neutral pH; enzymatic cleaner which is safe to use on delicate medical instruments; dissolves protein, mucous, and organic matter.

TABLE 2 presents a summary of product label information, including ingredients, excipients when listed and label statements. Proteolytic enzymes are listed as ingredients in all but two products. Three of the products list amylase as a ingredient in addition to a protease. These three do not list surfactants as additional ingredients. None of the products list lipase as an ingredient. Metrex MetriZyme, emPower and Sklar Enzymatic ™ are the only products listing surfactants as additional ingredients. A surfactant is critical to optimal cleaning performance. Metrex enzyme products are the only precleaners that claim a high level of proteolytic enzyme activity combined with surfactants. TABLE 2 notes that Endozime from Ruhof lists 10% isopropyl alcohol as an excipient. This will help to solubilize lipids. However, such solvents can inactivate proteolytic enzymes, and in fact, Endozime has nearly the lowest proteolytic enzyme activity of all enzymes tested.

TABLE 3 Comparative Foam Height



Low foaming characteristics are particularly desirable when using the solutions with automatic reprocessors as high foaming products have been shown to damage pumps. Three of the products claim to be low foaming: MetriZyme, emPower and Asepti-zyme ® from Huntington. TABLE 3 shows the results of a comparative foam height study. Although Asepti-zyme ® claims to be low foaming, it produced the third highest foam of all enzymes tested, whereas MetriZyme and produced a relatively low level of foam. The low foaming from emPower is attained through proprietary advanced technology. When compared to other available enzymatic products in the market, which contain surfactants, new emPower discloses a minimal foaming behavior while maximizing its cleaning capacity.

TABLE 4 pH Measurements of the Fully Diluted Products

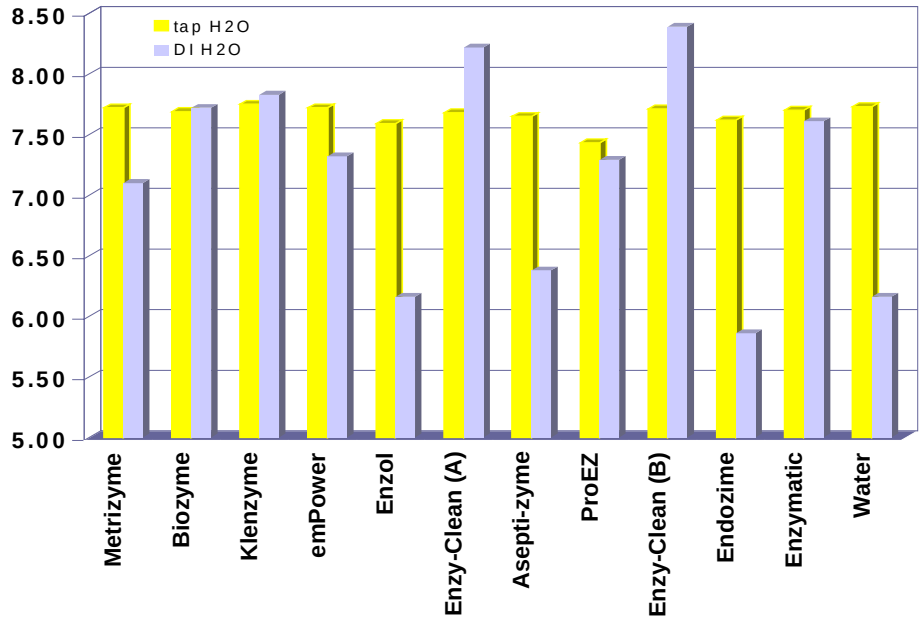
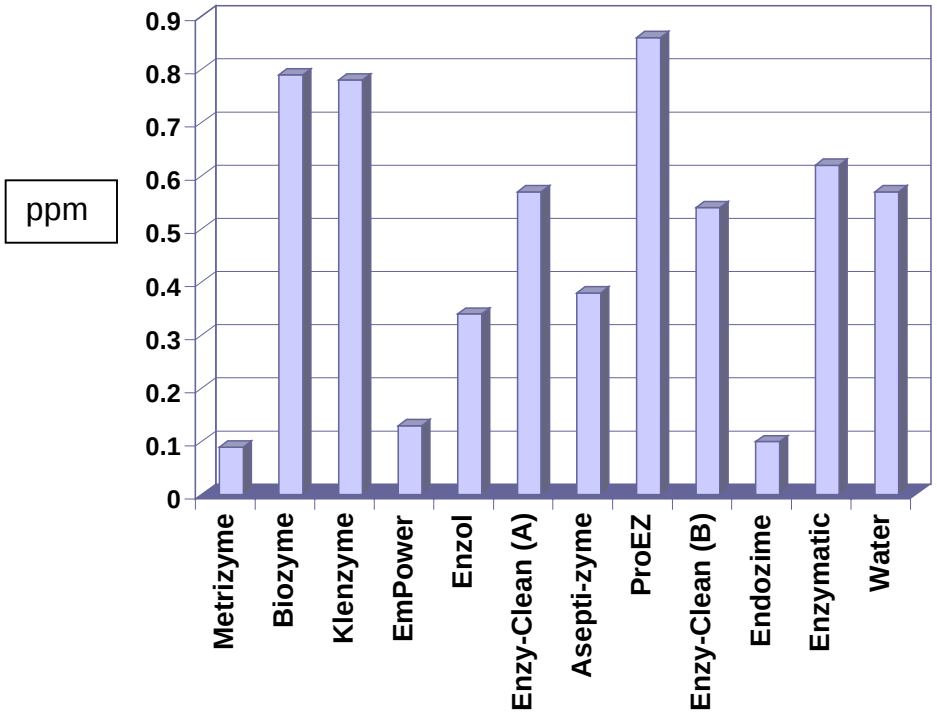


TABLE 4 presents pH measurements of the fully diluted products. All are very close to one another. An additional observation noted in the study was that only three products, MetriZyme, emPower and ProEz ™ from Cottrell, have a significant color when diluted. The coloring in these products was sufficient to indicate presence without adverse effect.

TABLE 5 Analysis of Corrosion of Stainless Steel



Corrosion is a major consideration when selecting a product for use with medical instrumentation. TABLE 5 presents an analysis of corrosion of stainless steel with various enzymatic detergents. Each product was diluted 1 ounce per gallon. 50mL of 1:128 diluted solution was exposed to stainless steel. The results clearly show that MetriZyme, emPower, and Endozime were the least corrosive enzymatic detergents tested and were significantly less corrosive than deionized water. For comparison purposes, a sample of chemical sterilants was also evaluated in the same manner. Cidex PA sterilant was found to have a value of 3.5 ppm, making it almost 6 times more corrosive than deionized water. The manufacturer is currently reformulating Cidex PA because it has been found to be too corrosive to instrumentation[ii]. This reinforces the fact that corrosion is a major issue in the marketplace.

In summary, this comparative assessment of commercially available enzyme-based medical instrument precleaners indicates that both MetriZyme and emPower are superior products with the highest proteolytic enzyme activity combined with surfactants and the lowest corrosion rates.

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[i] Martin, MA, Reichelderfer, M, APIC Guideline for Infection Prevention and Control in Flexible Endoscopy, *Am J Infect Control*, 1994;22:19-38

[ii] Rutala WA, Weber, DJ, Disinfection of Endoscopes: Review of New Chemical Sterilants Used for High- Level Disinfection, *Inft Control Hosp Epidemiol*, 1999, 20:74