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# *NovaStreak*

***for transport, screening, isolation and identification of bacterial contamination in food and dairy products***

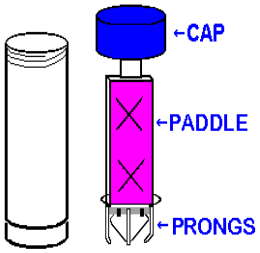
## INTENDED USE

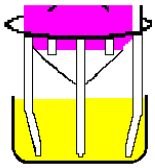
***NovaStreak*** is a convenient device for transporting samples as well as for isolating, enumerating and identifying specific bacteria in food with no dilution. A unique streaking mechanism permits the isolation of single colonies even when the original bacterial population of the sample was as high as $10^7$ organisms per milliliter. ***NovaStreak*** is intended for use in the food industry.

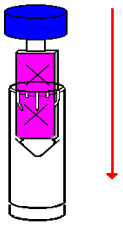
## SUMMARY AND EXPLANATION

***NovaStreak*** comprises a plastic paddle with two types of agar attached back-to-back, housed in a closed transparent plastic tube. A ring with elongated prongs is attached to the end of the paddle so that there are prongs on each side of the slide. The ends of the prongs are dipped into the undiluted sample. Upon re-insertion into the plastic tube, the prongs are prevented from moving and the agar surfaces are inoculated with bacteria as the paddle passes over the prongs. The result is a series of streaks of decreasing bacterial concentration that permits isolation of single colonies even when the original bacterial population of the sample was as high as $10^7$ organisms per milliliter.

## PROCEDURE





**1.** Unscrew the ***NovaStreak*** cap. Pull the paddle out. Do not touch any part <u>but</u> the cap.

**2.** Hold the paddle vertically and dip the white prongs into the sample up to about half of their length (see below).

**3.** Return the paddle to its container in a quick, continuous and vertical motion and tighten cap.

**4.** Transport the tube to laboratory for incubation and examination.

**5.** Before incubation loosen cap one-half turn.

**6.** Incubate the entire container in a vertical position as described for each media.

**7.** Determine the number of colony forming units by comparison to the Colony Density Chart.

## REAGENTS

| | | |
|---|---|---|
| **BD-504** | **Side 1:** Plate Count Agar (PCA)<br>**Side 2**: Oxytetracycline-Glucose-Yeast- Extract Agar (OGYE) | Agar Color: Yellow |
| **BD-506** | **Side 1:** Violet Red Bile Agar (VRBA)<br>**Side 2**: Oxytetracycline-Glucose-Yeast- Extract Agar (OGYE) | Agar Color: Purple<br>Agar Color: Yellow |
| **BD-507** | **Side 1:** Violet Red Bile Agar (VRBA)<br>**Side 2:** Plate Count Agar (PCA) | Agar Color: Purple<br>Agar Color: Yellow |
| **BD-508** | **Side 1:** Baird Parker Agar<br>**Side 2:** Violet Red Bile Agar (VRBA) | Agar Color: Yellow, Opaque<br>Agar Color: Purple |
| **BD-509** | **Side 1:** Baird Parker Agar<br>**Side 2:** Mannitol Salt Agar | Agar Color: Yellow, Opaque<br>Agar color: Red |
| **BD-512** | **Side 1:** KF Streptococcus Agar<br>**Side 2:** XLD | Agar Color: Yellow<br>Agar Color: Orange |
| **BD-519** | **Side 1:** XLD<br>**Side 2:** XLD | Agar Color: Orange |
| **BD-520** | **Side 1:** Chromocult<br>**Side 2:** PCA | Agar Color: Yellow |
| **BD-521** | **Side 1:** Chromocult<br>**Side 2:** Chromocult | Agar Color: Yellow |
| **BD-522** | **Side 1:** ChromoSal<br>**Side 2:** ChromoSal | Agar Color: Old Pink |

| REAGENTS | EXPECTED RESULTS |
|---|---|
| **Violet Red Bile Agar (VRBA)** is a selective medium for the detection of coliform organisms in water, milk, and other materials of sanitary importance. The medium is selective due to the presence of inhibitors, bile salts and crystal violet. Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink-to-red colonies are produced depending on the ability to ferment lactose. | Lactose fermenters are rose-red in color and generally surrounded by a halo of precipitated bile. *E. coli* colonies are entire edged, 1mm or more in diameter. *E. aerogenes* are larger, often mucoid and pinkish. Lactose non-fermenters produce colorless colonies. Enterococci occasionally grow to produce rose colonies pinpoint in size |
| **Oxytetracycline-Glucose-Yeast-Extract Agar (OGYE)** is recommended for the selection and enumeration of yeasts and molds from foodstuffs. The medium uses oxytetracycline as the selective agent at a neutral pH, that gives increased counts of yeasts and molds compared with media which rely on a low pH to suppress bacterial growth. | OGYE agar, after 5 days at 22 $\pm$ 3 $^{o}$ C produces good to excellent growth of *Aspergillus niger*, *Saccharomyces cerevisiae*, with total inhibition of *E. coli*. |
| **Baird Parker Agar** is used for the selective isolation and enumeration of coagulase-positive staphylococci from food, skin, soil, air and other materials. It may also be used to identify staphylococci on the basis of their ability to clear egg yolk (lecithinase production). Sodium pyruvate is incorporated to impart *S. aureus* their black colony appearance. Glycine and lithium chloride have inhibitory action on organisms other than *S. aureus.* | Typical colonies of *S. aureus* are black, shiny, convex and surrounded by clear halos of approximately 2 to 5 mm. Coagulase-negative staphylococci do not grow well; if at all, the typical clear halos are absent . Other organisms are inhibited or may grow sparsely, producing white to brown colonies with no clearing of the egg yolk. |
| **Mannitol Salt Agar** is used for the selective isolation and enumeration of staphylococci from clinical and non-clinical materials. The 7.5% sodium chloride concentration gives partial or complete inhibition of bacterial organisms other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator | *S. aureus* colonies have yellow zones after 48 hours. *S. epidermidis* have red zones after 48 hours. |

| REAGENTS | EXPECTED RESULTS |
|---|---|
| **KF Streptococcal Agar** is used to detect enterococci in surface waters. Azide suppresses the growth of Gram-negative microorganisms. Enterococci take up the vital stain triphenyltetrazolium chloride (TTC). | Enterococci produce pink-to-red colonies. |
| **Xylose Lysine Decarboxylase (XLD) Agar** is recommended for the isolation and differentiation of enteric pathogens, especially *Shigella*. Differentiation of *Shigella* and *Salmonella* from non-pathogenic bacteria is accomplished by three reactions: xylose fermentation, lysine decarboxylation, and hydrogen sulfide production. | *E. coli* and non-pathogenic coliforms may be partly inhibited or show large, flat, yellow colonies. *Shigella* produce red colonies, as do hydrogen sulfide-negative salmonellae. Hydrogen sulfide producing salmonella grow as red colonies with black centers. |
| **Plate Count Agar (PCA)** is also known as Standard Methods Agar. This medium contains casein hydrolyzate, dextrose and yeast extract. It is recommended for the isolation and enumeration of bacterial and fungal microorganisms of milk and other dairy products, and may be used to determine the sanitary quality of foods, water and other materials. | A variety of bacterial and fungal species appearing as contaminants in dairy and other food products will show good growth , including lactobacilli and staphylococci. |
| **ChromoSal (Salmon Gal) Agar** contains the chromogenic substrate beta-D-galactoside. | Beta-D-galactosidase-positive *Salmonella* produce blue colonies. |
| **Chromocult (X-Gluc) Agar** contains the chromogenic substrate X-beta-D- glucuronide. The substrate is also known as X-GLUC. | X-beta-D-glucuronidase-producing *E. coli* produce pink colonies. |

**Storage:** store ***NovaStreaks*** at 4-8°C out of direct light. May be used until expiration date shown on the label.

**Interpretation:** Preliminary identification of the bacteria can be made based on the type and color of the colonies. For example:

| | **VRBA** Agar Color: Purple | **OGYE** Agar Color: Yellow | **PCA** Agar Color: Yellow | **Baird Parker** Agar Color: Yellow, Opaque | **Mannitol** Agar color: Red | **KF** Agar Color: Yellow | **Midllebrook** Agar Color: Green | **XLD** Agar Color: Orange |
|---|---|---|---|---|---|---|---|---|
| ***E. coli* coliforms** | Red colonies | No growth | White colonies | No growth | No growth | No growth | | Partial growth, Yellow colonies |
| ***S. aureus*** | No growth | No growth | White colonies | Black colonies | Yellow colonies | No growth | | No growth |
| ***S. typhimurium*** | Colorless colonies | No growth | White colonies | | | No growth | | Transparent with black centers |
| ***E. faecalis*** | | | | | | Red colonies | | |
| ***S. epidermidis*** | | | | Grey-Black colonies | Red colonies | | | No growth |
| ***C. albicans*** | No growth | White colonies | White colonies | | | | | |
| ***M. tuberculosis*** | | | | | | | White colonies | |
| ***M. bovis*** | | | | | | | White colonies | |
| ***S. flexneri*** | | | | | | | | Red colonies |

## MICROBIOLOGICAL CRITERIA

Mandatory microbiological criteria have been established by such bodies as the U.S. Public Health Service, the USDA, the ICMSF, etc. [1-4]. Test parameters for raw milk call for an aerobic plate count using a plate count agar (PCA)/standard methods agar (STM). For Grade A Pasteurized (uncultured and cultured milk) aerobic bacteria and coliforms must be tested. Frozen desserts must be similarly tested for total aerobes and coliforms. Breaded shrimps must be tested for total aerobes, *E. coli*, *S. aureus*. In other countries caseins and caseinates must be tested for total aerobic microorganisms, thermophilic organisms and coliforms. Codex criteria for natural mineral waters include an aerobic count of mesophiles, coliforms, *E. coli*, enterococci, sulphite reducing anaerobes, *P. aeruginosa*, and other pathogenic organisms. In addition, Canadian criteria call for a mold count in tomato juice, a total plate count and *E. coli* count for fish protein, as well as a total plate count, coliform and Salmonella counts for gelatin.

Advisory microbiological criteria have also been established by ICMSF and the Codex Commission [5-6] for, among others, the following food products: Roast beef as well as pate should be tested for Salmonella. On raw chicken an aerobic plate count should be performed. Cooked poultry (frozen) should be tested for *S. aureus* and Salmonella. Fresh and frozen fish (to be cooked) should be tested for total aerobes, *E. coli*, *Vibrio parahaemolyticus*, *S. aureus* and Salmonella. Dried milk should be tested for total aerobes, coliforms and Salmonella (for normal and high risk populations). Fresh cheese and soft cheese should be tested for *S. aureus* and coliforms. Egg products (pasteurized liquid, frozen and dried) should be tested for total aerobes, coliforms, and Salmonella (for normal and high risk populations). Chocolate/confectionery, as well as coconut should be tested for Salmonella.

## References:


1. Public Health Service-Food and Drug Administration. 1978. Grade A Pasteurized Milk Ordinance. 1978 Recommendations. PHS/FDA Publication No. 229. U.S. Government Printing Office, Washington, D.C.
2. Department of Agriculture. 1975. General specifications for approved dairy plants and standards for grades of dairy products. Fed. Regist. 40(198):47910-47940.
3. Food and Drug Administration. 1989. Raw breaded shrimp-microbiological criteria for evaluating compliance with current good manufacturing practice regulations, chapter 8. In: Compliance Policy Guides 7108.25. Food and Drug Administration, Washington, D.C.
4. National Research Council. 1985. An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients. National Academic Press, Washington, D.C.
5. International Commission on Microbiological Specifications for Food. 1986. Microorganisms in Foods: Sampling for microbiological Analysis: Principles and Applications. 2nd ed. University of Toronto Press. Toronto.
6. Codex Alimentarius Commission, 20th session. 1993. Report of the 20th Session of the Codex Commission on Food Hygiene. Alinorm 93/13A. Food and Agriculture Administration, Rome.