SODIUM POLYANETHOL SULFONATE (SPS) IDENTIFICATION DISKS

Cat. no. Z7381	HardyDisk™ SPS Identification Disks	50 disks/cartridge

INTENDED USE

HardyDisk™ SPS Identification Disks evaluate the ability of an organism to grow in the presence of Sodium Polyanethol Sulfonate. HardyDisk™ SPS Identification Disks can be used to presumptively identify microorganisms including but not limited to *Peptostreptococcus anaerobius*, *Gardnerella vaginalis*, and *Haemophilus ducreyi*.

SUMMARY

Peptostreptococcus belong to the genus of gram-positive anaerobic cocci. These organisms are widely distributed as normal flora in humans and animals and account for up to a third of anaerobic human isolates.[6] The three species of *Peptostreptococcus* most commonly reported in clinical specimens are *P. anaerobius*, *P. magnus*, and *P. asaccharolyticus*. Specific identification of most anaerobic cocci requires both biochemical tests and gas-liquid chromatography, as these organisms tend to exhibit few visual phenotypic differences.[2]

In contrast to traditional testing procedures, the Sodium Polyanethol Sulfonate (SPS) disk test is a simple and rapid technique used for the presumptive identification of *P. anaerobius* from other gram-positive anaerobic cocci. The characteristic inhibition by SPS to differentiate *P. anaerobius* from other gram-positive anaerobic cocci was initially demonstrated by Graves, et al.[1] The SPS disk test has since been demonstrated to have an overall accuracy of 98% in the identification of *P. anaerobius*[9] Consequently, it is recommended that the SPS disk test be routinely applied to all gram-positive anaerobic cocci isolates to presumptively identify *P. anaerobius*.[8]

SPS inhibition is also useful in the presumptive identification of microorganisms other than *P. anaerobius*. Reimer and Reller initially demonstrated that SPS disk susceptibility and alpha-hemolytic streptococci inhibition can be employed to accurately identify *Gardnerella vaginalis*.[10] Currently the differentiation of *G. vaginalis* from other catalase-negative, gram-variable, coccobacilli is achieved solely on the susceptibility of *G. vaginalis* to SPS.[4] An additional study performed by Shawar, et al. confirmed that SPS susceptibility can also be used to differentiate *Haemophilus ducreyi* from other *Haemophilus* species.[11]

FORMULA

Each HardyDisk™ SPS Identification Disk is prepared by impregnating 1mg of Sodium Polyanethol Sulfonate onto a 0.25 inch diameter filter paper disk.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. The disks should not be used if there are any signs of deterioration or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, and moisture.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

1. Allow disks to equilibrate to room temperature before use. Prepare a suspension, equivalent to a 0.5 McFarland turbidity standard, of the organism to be tested from a pure 24-48 hour culture.

2. Dip a sterile non-toxic swab into the organism suspension. Rotate the swab several times, pressing firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. Evenly inoculate the dried surface of a Brucella Agar with Vitamin K and Hemin plate (Cat. no. A30) to obtain confluent growth.

3. Place one SPS disk on the media surface. With sterile forceps, gently tap the disk to ensure complete contact with the plate surface.

4. Incubate the media at 35ºC. for 24-48 hours. Consult listed references for recommended incubation atmosphere.[2-5]

5. After incubation, examine the media surface and measure the zone diameter surrounding the SPS disk. Zones should be recorded to the nearest whole millimeter.

INTERPRETATION OF RESULTS

When the zone diameter is greater than or equal to 12mm, the organism is considered susceptible to SPS. When the zone diameter is less than or equal to 11mm, the organism is considered resistant to SPS.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

P. micros and *P. prevotii* produce small zones of inhibition, usually less than 10mm. Occasionally isolates can produce zones larger than 12mm, which will be interpreted as sensitive.[8]

H. ducreyi is susceptible with a zone greater than or equal to 12mm; all other *Haemophilus* species are resistant and will have zones less than 12mm. *Capnocytophaga* is the only other genus of gram-negative rods that are susceptible to SPS, but they are ALA-positive. The addition of 0.002% Tween® 80 may aid in the dispersion of the cells.[4,11]

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Peptostreptococcus anaerobius ATCC® 27337	F	24-48hr	35°C	Anaerobic	Zone of inhibition greater than or equal to 12mm
Gardnerella vaginalis ATCC® 14018	F	24-48hr	35°C	CO_2**	Zone of inhibition greater than or equal to 12mm
Bacteroides fragilis ATCC® 25285	F	24-48hr	35°C	Anaerobic	Zone of inhibition less than 12mm

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

** Atmosphere of incubation is enriched with 5-10% CO_2.

PHYSICAL APPEARANCE

HardyDisk™ SPS Identification Disks are 0.25 inch (in diameter) filter paper disks with the letters SPS on both sides, and should appear white in color.

Showing SPS Sensitivity
Gardnerella vaginalis (ATCC® 14018) growing with a zone around a HardyDisk™ SPS Identification Disks (Cat. no. Z7381), demonstrating sensitivity. Incubated in CO_2 for 48 hours at 35ºC.

Showing SPS Resistance
Bacteroides fragilis (ATCC® 25285) growing around a HardyDisk™ SPS Identification Disks (Cat. no. Z7381), demonstrating resistance. Incubated anaerobically for 48 hours at 35ºC.

REFERENCES

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IFU-10737[A]

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
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HDQA 2207F [D]