

SPS Agar

Intended Use

SPS Agar is used for detecting and enumerating *Clostridium perfringens* in food.

Summary and Explanation

In the 1950s, Mossel¹ and Mossel et al.² proposed media for enumerating anaerobic sulfite-reducing clostridia in foods. Angelotti et al.³ modified the formula as Sulfite Polymyxin Sulfadiazine (SPS) Agar and used it to quantitate *C. perfringens* in foods.

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients. Occurrences of foodborne illness from *C. perfringens* are usually associated with cooked meat or poultry products.⁴ Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.⁵ Enumerating the microorganism in food samples plays a role in epidemiological investigation of outbreaks of foodborne illness.⁴

Principles of the Procedure

SPS Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Ferric citrate and sodium sulfite are H₂S indicators. Clostridia reduce the sulfite to sulfide, which reacts with the iron from ferric citrate to form a black iron sulfide precipitate. Polysorbate 80 is a dispersing agent. Polymyxin B sulfate and sulfadiazine are inhibitors to organisms other than *Clostridium* spp. Sodium thioglycollate is a reducing agent. Agar is the solidifying agent.

Formula

Difco™ SPS Agar

Approximate Formula* Per Liter

Tryptone	15.0	g
Yeast Extract	10.0	g
Ferric Citrate	0.5	g
Sodium Sulfite.....	0.5	g
Sodium Thioglycollate	0.1	g
Polysorbate 80	0.05	g
Sulfadiazine	0.12	g
Polymyxin B Sulfate.....	0.01	g
Agar	15.0	g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 41 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Dispense inoculum into sterile Petri dish.
2. Pour medium cooled to 50-55°C over the inoculum.
3. Gently but thoroughly mix the inoculum and medium. Allow to solidify on a flat surface.
4. Incubate anaerobically at 35 ± 2°C for 24-48 hours.

Expected Results

Clostridium perfringens will grow as black colonies with good growth.

User Quality Control

Identity Specifications

Difco™ SPS Agar

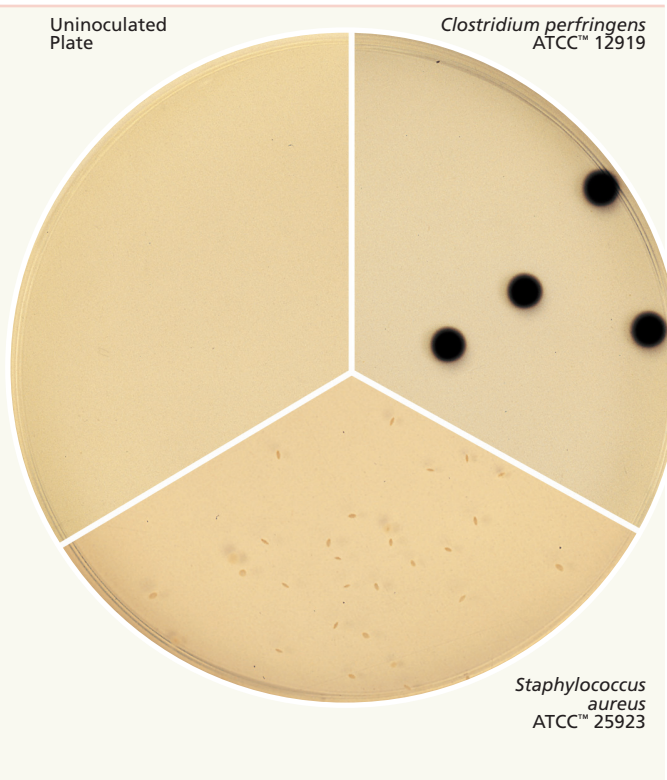
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.1% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ SPS Agar

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate anaerobically at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Clostridium perfringens</i>	12919	10 ² -10 ³	Good	Black
<i>Clostridium sporogenes</i>	11437	10 ² -10 ³	None to fair	Black
<i>Escherichia coli</i>	25922	10 ² -10 ³	Marked to complete inhibition	—
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ² -10 ³	Marked to complete inhibition	—
<i>Staphylococcus aureus</i>	25923	10 ² -10 ³	Fair to good	White



Limitation of the Procedure

The high degree of selectivity of SPS Agar may inhibit some strains of *C. perfringens* while other strains that grow may fail to produce distinguishing black colonies.⁴

References

1. Mossel, 1959. J. Sci. Food Agric. 19:662.
2. Mossel, DeBruin, van Diepen, Vendrig and Zoutewelle. 1956. J. Appl. Microbiol. 19:142.
3. Angelotti, Hall, Foster and Lewis. 1962. Appl. Microbiol. 10:193.
4. Labbe. 2001. In Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
5. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

Availability

Difco™ SPS Agar

Cat. No. 284530 Dehydrated – 500 g*

*Store at 2-8°C.