



LBM®
Selenite

Per poter eseguire la semina, seguire la procedura descritta per la semina dei brodi riportata sul manuale dell'automazione WASP[™].

CONTROLLO QUALITA'

VERIFICA DI ARRICCHIMENTO

- Da una coltura fresca di *Salmonella enterica* ATCC 14028, preparare un inoculo 0.5 Mac Farland in PBS.
- Preparare una diluizione in serie 10⁻⁵ dallo 0,5 Mac Farland in modo da ottenere una conta in piastra compresa fra 30 e 300 cfu/100 µL.
- Aprire la provetta di Selenite Broth sotto cappa a flusso laminare.
- Con una micropipetta inoculare 200 µL di sospensione direttamente nella provetta di Selenite Broth da 2 ml (o 500 µL in quella da 5 mL).
- Ritappare la provetta.
- Vortexare per 10 secondi a 2000/2500 rpm al minuto.
- Riaprire immediatamente il tubo e piastare 100 µL su una piastra di agar XLD o come da procedura interna al laboratorio NOTE: La piastra deve essere siglata come tempo zero del test.
- Incubare il tubo a 35°C ± 2°C per 18-24 ore.
- Dopo l'incubazione, piastare 100 µL di Selenite Broth su una piastra di agar XLD o come da procedura interna al laboratorio, su un altro terreno adatto all'isolamento *Salmonella* spp.
- Incubare la piastra di agar come previsto nel procedimento standard di laboratorio, a 35°C ± 2°C per 18-24 ore.

Il conteggio al tempo zero deve essere 30-300 cfu/piastra e la piastra dopo un periodo di 18-24 ore deve mostrare crescita confluyente.

PROVA DI INIBIZIONE:

- Da una coltura fresca di *E.coli* ATCC 25922, preparare un inoculo pari a 0,5 Mac Farland in PBS.
- Aprire sotto cappa a flusso laminare la provetta di Selenite Broth.
- Con una micropipetta inoculare 200 µL di inoculo direttamente nella provetta di Selenite Broth da 2 ml (o 500 µL in quella da 5 mL).
- Ritappare la provetta.
- Vortexare per 10 secondi a 2000/2500 rpm al minuto.
- Riaprire immediatamente la provetta e piastare 100 µL su una piastra di agar McConkey o come da procedura interna al laboratorio su un altro terreno adatto all'isolamento di *E.coli*. La piastra deve essere siglata come tempo zero del test.
- Incubare a 35°C ± 2°C per 18-24 ore.
- Dopo l'incubazione, seminare 100 µL di Selenite Broth su una piastra di agar McConkey o come da procedura interna al laboratorio, su un altro terreno adatto all'isolamento di *E.coli*.
- Incubare la piastra di agar come previsto nel procedimento standard di laboratorio, a 35°C ± 2°C per 18-24 ore.

Il conteggio al tempo zero deve mostrare una crescita confluyente e la piastra dopo un periodo di 18-24 ore deve mostrare un'inibizione parziale o completa.

Ceppi	Risultati cfu/piastra tempo zero Media su 5 piastre	Risultati cfu/piastra tempo 24 ore Media su 5 piastre
<i>Salmonella enterica</i> Serovar Typhimurium ATCC 14028	172	CRESCITA CONFLUENTE
<i>E.coli</i> ATCC 25922	CRESCITA CONFLUENTE	INIBIZIONE DA PARZIALE A TOTALE
<i>Shigella sonnei</i> ATCC 9290	202	CRESCITA CONFLUENTE

ENGLISH

Copan SELENITE Information Leaflet & User Guide

See the glossary of symbols at the end of the information leaflet

INTENDED USE

Copan **Selenite Broth** is a selective enrichment broth for clinical samples used for the culturing and the subsequent isolation of enteric pathogens including *Salmonella* spp. and *Shigella* spp.

SUMMARY AND PRINCIPLES

Diarrhoea can be defined as an increased frequency of bowel movements with at least three discharges a day accompanied by a decreased stool consistency. It is caused by a wide range of pathogens (bacteria, viruses and parasites) through different pathogenic mechanisms. *Salmonella* spp. and *Shigella* spp. infections come from the multiplication of intestinal bacteria. Most *Salmonella* spp. infections in humans are related to the consumption of animal based food, water contaminated by animals or interpersonal contact. Gastroenteritis is the most common infection that occurs. A small amount of *Salmonella* spp. may be present in the faeces of asymptomatic carriers. *Shigella* spp. infections have a wide variety of symptoms that range from mild diarrhoea to dysentery. Shigellas are transmitted directly from person to person, environmental multiplication is insignificant and asymptomatic infections are rare⁽²⁾. The aetiological diagnosis of *Salmonella* spp and *Shigella* spp diarrhea is done by seeding the fecal sample directly onto a moderately selective solid culture medium such as Hektoen enteric agar/XLD (Xylose lysine deoxycholate), a more selective medium such as SS (Salmonella Shigella Agar)^(1,2) or by passing from an enrichment phase in a liquid medium such as Selenite broth^(1,2). Using an enrichment broth can increase the recovery of *Salmonella* spp and *Shigella* spp by 10% and is particularly recommended in populations such as service personnel and nutritionists⁽⁵⁾.

PRODUCT DESCRIPTION

Catalogue number.	Product description	Package dimensions
475CE.A	Polypropylene 12x80 mm skirted screw-cap tube with internal conical shape filled with 2 ml of Selenite broth.	50 units in each sales pack 6x50 units per box
475CE	Polypropylene 12x80 mm skirted screw-cap tube with internal conical shape filled with 2 ml of Selenite broth.	50 units per sales pack 6x50 units per case
4E021N.A	Polypropylene 12x80 mm round bottom tube with screw-cap filled with 2 ml of Selenite broth.	50 units per sales pack 6x50 units per case
082CU.A	Polypropylene 16x100 mm skirted screw-cap tube with internal conical shape filled with 5 ml of Selenite broth.	50 units per sales pack 6x50 units per case

FORMULA

Peptone
Lactose
Sodium phosphate salt
Sodium selenite salt

REQUIRED MATERIALS BUT NOT INCLUDED

Suitable materials for the isolation and culturing of bacteria; material for patient sample collection (different types of collection and transport systems, with Amies transport medium i.e. eSwab/FecalSwab).
Refer to the laboratory reference manuals for the recommended culture techniques and identification procedures.

PRODUCT STORAGE

This product is ready for use and requires no further preparation. It must be stored in its original container at a temperature between 5° and 25°C until the time of use. Do not overheat. Do not incubate or freeze prior to use. Improper storage can cause a loss of effectiveness. Do not use after the expiration date, which is clearly printed on the outside of the box and on the test tube label.

LIMITATIONS

- The sample collecting conditions, times and volume for the culture are important variables for obtaining reliable culture results. Follow the recommended sample collection guidelines.
- The performance tests on the Copan Selenite broth were performed using ATCC strains and not using human clinical samples.
- An adequate collection of samples from the patient is an extremely critical factor for the successful isolation and identification of infectious organisms. Consult the published reference manuals for specific guidance on the sample collecting procedures. Samples should be collected as soon as possible after clinical signs of the disease appear. Higher bacterial concentrations are present during the acute phase of the disease⁽⁷⁾.
- Selenite Broth is a selective and enrichment broth for the isolation of samples of *Salmonella spp* and *Shigella spp*. The product was not designed for the enrichment of other genera and species of bacteria.
- Fecal samples require special attention. They must be taken in the early stages of the disease and must be cultured within 2 hours after collection. Under optimal conditions, the sample should be transferred into the transport medium immediately after collection (products such as the Copan Fecal Swab and Copan Cary Blair are suggested sample transport systems) in order to keep the organisms viable. Samples should never be refrigerated⁽⁷⁾.
- There is no dulcitol (melampirite or polyalcohol) in the Copan Selenite Broth therefore the growth of coliforms could be suppressed during the first 8-12 hours of incubation; longer incubation times may result in the excessive growth of mixed flora⁽⁸⁾.

WARNINGS AND PRECAUTIONS FOR USE

- Observe approved biohazard precautions and aseptic techniques.
- The product is only to be used by adequately trained and qualified personnel.
- All samples and the materials used to process them must be considered potentially infective and must be handled in such a way as to prevent infection of the laboratory staff.
- Observe the other applicable biosafety recommendations.
- Carefully read and follow the instructions.
- For *in vitro* diagnostic use. This product must not be used for therapeutic or prophylactic purposes.
- Carefully read and follow the instructions.
- ⚠ This product is intended for single use; if reused, there is the risk of obtaining misleading results.
- Not suitable for any other application different from its intended use.
- Work under a biosafe laminar flow hood. Wear latex gloves and other protection commensurate with universal precautions when handling clinical specimens.
- The use of this product in association with any diagnostic tests or any diagnostic instrumentation should be previously evaluated by the user.
- Do not use the product if it is visibly damaged.
- The liquid contained in the individual tubes is TOXIC. Do not swallow or inhale and avoid contact with the skin and eyes. Take appropriate protective measures.
- All samples are to be considered potentially infectious and must be handled with appropriate precautions.
- The product is only to be used by properly trained and qualified personnel.
- ⚠
H302: Harmful if swallowed.
EUH031: Contact with acids liberates toxic gas.
EUH208: Contains sodium selenite. May produce an allergic reaction.

PRODUCT DETERIORATION

Do not use Copan Selenite Broth if: 1. the product has visible signs of damage or contamination; 2. there is evidence of liquid leaking from the test tube; 3. it has passed the expiration date; 4. there are other visible signs of deterioration.

INSTRUCTIONS FOR USE

Use in the laboratory

Selenite Broth is used for the selective enrichment of *Salmonella* spp and *Shigella* spp present in fecal or rectal samples. The Selenite Broth comes with a special cap that has an adapter for attaching the shaft of the swab used during sampling (sampling swabs with a marked break point on the shaft are recommended for use with the Selenite Broth tubes. For further information please contact Copan Italia S.p.A.). If a rectal or fecal swab is obtained using the FecalSwab collection and transport system, this swab can be transferred into the Selenite Broth as indicated in the procedure below.

1. Unscrew the cap of the Selenite Broth tube.
2. Add the sample as described below:

Rectal or fecal swab: (homogenize the sampling system with the sample by vortexing the test tube containing the sample for 10 seconds at 2000-2500 rpm).

- For a rectal or fecal swab obtained using FecalSwab: open the tube and transfer the swab into the Selenite Broth using sterile tweezers; alternatively, transfer approximately 30µl of culture medium in the FecalSwab system using a micropipette; or transfer the shaft attached to the cap of the FecalSwab system directly into the Selenite Broth, then close the FecalSwab tube with the cap from the Selenite tube.
- For a generic rectal or fecal swab or a dry swab (without transport medium): break the swab at the marked break point inside the Selenite Broth and close the cap, or vigorously shake the swab without a break point in the tube for at least 10 seconds.
- Close the inoculated Selenite Broth tube.
- Recap the tube/container with the original sample.

Fecal sample:

- Add a portion of the fecal sample using a swab or a special scoop.

NOTE: make sure that the swab used to transfer the feces is completely covered with fecal material.

- Transfer a portion of the feces into the Selenite Broth. Follow the guidelines used in your laboratory for the volumetric ratio.
- Vigorously shake the swab or scoop in the Selenite Broth for at least 10 seconds, in order to release the collected material.
- Close the Selenite Broth tube.

3. Homogenize the tube by vortexing it for 10 seconds at 2000-2500 rpm;
4. Incubate the Selenite Broth tubes at 35°C ± 2°C for 18-24 hours;
5. After 18-24 hours look for any turbidity in the broth. If negative, incubate for another 18-24 hours;
6. Inoculate a plate of selective and/or differential growth media with 1 to 10 µl of enriched broth.

The enriched sample in Selenite Broth can be seeded with automated sample management systems such as WASP™/WASPLab™. For seeding, follow the procedure for seeding broths given in the WASP™ automation manual.

QUALITY CONTROL

ENRICHMENT VERIFICATION

- Prepare a 0.5 Mac Farland inoculum in PBS from a fresh culture of *Salmonella enterica* ATCC 14028.
- Prepare a 10⁻⁵ in series dilution from the 0.5 Mac Farland in order to obtain a plate count of between 30 and 300 CFU/100 µL.
- Open the tube of Selenite Broth under a laminar flow hood.
- Using a micropipette, inoculate 200 µL of the suspension directly in the 2 ml tube of Selenite Broth (or 500 µL in a 5 mL tube).
- Recap the tube.
- Vortex for 10 seconds at 2000-2500 rpm.
- Immediately re-open the tube and streak an XLD agar plate with 100 µL or following the internal laboratory procedure. NOTE: The plate must be marked as time zero for the test.
- Incubate the tube at 35°C ± 2°C for 18-24 hours.
- After incubation, streak an XLD agar plate with 100 µL of Selenite Broth or following the internal laboratory procedure using another type of culture medium suitable for isolating *Salmonella* spp.
- Incubate the agar plate as required in the standard laboratory procedure, at 35°C ± 2°C for 18-24 hours.

The plate count at time zero must be 30-300 CFU/plate and confluent growth must be visible after 18-24 hours.

INHIBITION TEST:

- Prepare a 0.5 Mac Farland inoculum in PBS from a fresh culture of *E.coli* ATCC 25922.
- Open the tube of Selenite Broth under a laminar flow hood.
- Using a micropipette, inoculate 200 µL of the inoculum directly in the 2 ml tube of Selenite Broth (or 500 µL in a 5 mL tube).
- Recap the tube.
- Vortex for 10 seconds at 2000-2500 rpm.
- Immediately re-open the tube and streak a plate of McConkey agar with 100 µL or following the internal laboratory procedure using another type of culture medium suitable for isolating *E.coli*. The plate must be marked as time zero for the test.
- Incubate at 35°C ± 2°C for 18-24 hours.
- After incubation, streak a McConkey agar plate with 100 µL of Selenite Broth or following the internal laboratory procedure using another type of culture medium suitable for isolating *E.coli*.
- Incubate the agar plate as required in the standard laboratory procedure, at 35°C ± 2°C for 18-24 hours.

The plate must show confluent growth at time zero and partial or complete inhibition must be visible after 18-24 hours.

Strains	Results CFU/plate at time zero	Results CFU/plate after 24 hours
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	Average of 5 plates	Average of 5 plates
<i>Salmonella enterica</i> Serovar Typhimurium ATCC 14028	172	CONFLUENT GROWTH
<i>E.coli</i> ATCC 25922	CONFLUENT GROWTH	PARTIAL TO TOTAL INHIBITION
<i>Shigella sonnei</i> ATCC 9290	202	CONFLUENT GROWTH

ESPAÑOL

Copan SELENITE Folleto ilustrativo y guía de uso

Véase el glosario de símbolos al final del folleto ilustrativo.

USO PREVISTO

Copan **Selenite Broth** es un caldo selectivo de enriquecimiento para muestras clínicas previsto para el cultivo y el posterior aislamiento de patógenos entéricos, incluyendo *Salmonella* spp. y *Shigella* spp.

INTRODUCCIÓN Y PRINCIPIOS

La diarrea puede definirse como una frecuencia aumentada de la actividad intestinal con al menos tres descargas diarias acompañadas de una reducida consistencia de las heces. Está causada por un amplio número de patógenos (bacterias, virus y parásitos) mediante diversos mecanismos de patogenicidad. En el caso de las infecciones por *Salmonella* spp. o *Shigella* spp., es el resultado de la multiplicación bacteriana en el intestino. La mayor parte de las infecciones humanas por *Salmonella* spp. están asociadas al consumo de alimentos de origen animal, al agua contaminada con animales o al contacto interpersonal. La gastroenteritis es la infección más común. Un bajo número de *Salmonella* spp. puede estar presente en las heces de portadores asintomáticos. Las infecciones por *Shigella* spp. se manifiestan con un amplio rango de síntomas, desde una ligera diarrea hasta la disentería. Las shigelas se transmiten directamente de persona a persona, la multiplicación ambiental es poco relevante y son raras las infecciones asintomáticas ⁽²⁾. El diagnóstico etiológico de diarrea por *Salmonella* spp. y *Shigella* spp. se lleva a cabo sembrando la muestra fecal directamente en un medio sólido moderadamente selectivo, como Hektoen Enteric Agar/XLD (agar de xilosa, lisina, desoxicolato), en un medio más selectivo, como SS (Salmonella Shigella Agar) ^(1,2), o bien pasando por una fase de enriquecimiento en un medio líquido, como Selenite broth ^(1,2). El uso de un caldo de enriquecimiento puede aumentar la recuperación de *Salmonella* y *Shigella* spp. un 10 %, y se recomienda especialmente en poblaciones como personal de asistencia o tenderos ⁽⁵⁾.

DESCRIPCIÓN DEL PRODUCTO

Número de catálogo	Descripción del producto	Tamaño del paquete
475CE.A	2 ml de caldo Selenite en probeta de polipropileno de 12x80 con tapón de rosca, interior con forma cónica, se mantiene de pie.	50 unidades por envase comercial 6x50 unidades por caja
475CE	2 ml de caldo Selenite en probeta de polipropileno de 12x80 con tapón de rosca, interior con forma cónica, se mantiene de pie.	50 unidades por envase comercial 6x50 unidades por caja
4E021N.A	2 ml de caldo Selenite en probeta de polipropileno de 12x80 con tapón de rosca y forma redondeada, NO se mantiene de pie.	50 unidades por envase comercial 6x50 unidades por caja
082CU.A	5 ml de caldo Selenite en probeta de polipropileno de 16x100 con tapón de rosca, interior con forma cónica, se mantiene de pie.	50 unidades por envase comercial 6x50 unidades por caja

FÓRMULA

Peptona
Lactosa
Sal de fosfato de sodio
Sal selenito de sodio

MATERIAL NECESARIO PERO NO INCLUIDO

Materiales adecuados para el aislamiento y el cultivo de bacterias; material para recogida de muestras del paciente (diferentes tipos de recogida y transporte suministrados o no, con medio de transporte Amies, es decir, eSwab/FecalSwab).

Consultar los manuales de referencia del laboratorio para ver los protocolos recomendados para las técnicas de cultivo y de identificación.

CONSERVACIÓN DEL PRODUCTO

Este producto está listo para el uso y no requiere ningún tipo de preparación. El producto debe conservarse en el embalaje original a una temperatura de 5 a 25 °C hasta el momento en que se va a utilizar. No sobrecalentar. No incubar ni congelar antes de usar. Si se guarda de forma incorrecta puede perder su eficacia. No usar después de la fecha de caducidad que aparece claramente impresa en el exterior de la caja y en la etiqueta de la probeta.

LÍMITES DE USO

1. Las condiciones, el tiempo y el volumen de la muestra recogida para el cultivo son variables importantes para garantizar resultados de cultivo fiables. Observar las directrices recomendadas para la recogida de muestras.