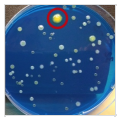

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Isolation of Klebsiella strains from human or animal fecal material

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1 Works for me dx.doi.org/10.17504/protocols.io.662hhge

Klebsiella Research and Surveillance

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ABSTRACT

This protocol is intended for isolation of Klebsiella strains from human or animal fecal material. It is derived from the initial description of the SCAi medium (van Kregten E, Westerdaal, N. A. C., and Willers, J. M. N. New, simple medium for selective recovery of Klebsiella pneumoniae and Klebsiella oxytoca from human feces. Journal of Clinical Microbiology. 1984;20:936-41) and its validation across a diversity of Klebsiella strains (Passet V, Brisse S. 2015. Association of tellurite resistance with hypervirulent clonal groups of Klebsiella pneumoniae. J Clin Microbiol. 53(4):1380-2). The protocol entails enrichment using ampicillin-containing broth, and plating on SCAi (Simmons Citrate with Inositol) agar.

GUIDELINES

There is no commercial availability of SCAi agar plates (in 2019). Plates must be prepared locally and can be stored several weeks at 4°C

MATERIALS

NAME	CATALOG #	VENDOR
Ampicillin	A9518-5g	Sigma-aldrich
myo-inositol	I5125-50 g	Sigma Aldrich
Simmons citrate	64834-500g	BioRad Sciences

MATERIALS TEXT

1. Ampicillin stock solution preparation:

Preparation for [10 mg/mL] in 10 mL of H2O

Calculations:
10 mg x 10ml =100 mg = 0,1 g
371.39 g of powder ------- 348.39 g of ampicillin
X ---------- 0.1g
X= 0.107 g (107 mg) of ampicillin sodium salt powder

Steps:
Weigh 0.107 g of **ampicillin sodium salt powder** and dissolve in 10 mL of water.
Sterilize by filtration (0.22 µm syringe filter).
Store the ampicillin in aliquots at -20°C for 1 year (or at 4°C for 3 months).
Use at final concentration of 10 microgram/mL (0.01 mg/mL) in LB (or TCS) broth.

Example:

For 10 mL of LB medium add 10 microL of ampicillin stock solution

2. Myo-inositol solution preparation

Catalog: Sigma-Aldrich I5125-50 g

Preparation of myo-inositol at 10 %

Steps:
Weigh 10 g of myo-inositol and dissolve in 100 ml of water.
Sterilize by filtration (as for ampicillin).

3. Simmons Citrate Agar

Catalog: Bio-rad 64834-500g or Dutscher 777388-500g

For Simmons citrate agar from BioRad:

Steps:
Suspend 21 g of the powder in 1 liter of purified water. Mix thoroughly.
Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
Autoclave at 121°C for 20 minutes.
Cool to 45-55 °C and take 900 ml Simmons Citrate Agar
Add 100 ml myo-inositol at 10 % (leading to a final concentration of 1%)
Pour into sterile Petri plates and store at 4 °C.

Note: Simmons citrate can also be ordered at Conda (ref. Simmons Citrate agar ISO 10273, catalog Number 1014). In that case 24.3 g of powder must be used for one liter, instead of 21 g.

BEFORE STARTING

Prepare ampicillin solution and SCAi agar plates

24h

1 **Pre-treatment of sample**

Enrich the sample by use of an enrichment broth.

We typically use **Luria-Bertani broth with 10 mg/L of ampicillin final concentration.** Because they carry a constitutively expressed class A beta-lactamase (blaSHV type), the vast majority of Klebsiella isolates will grow/survive, whereas many other bacteria including E. coli (the most abundant potential competitor) are susceptible to ampicillin. Trypto-Casein Soy broth (TSB) can be used instead of LB.

Take a swab or a loopful (10 µL) of faecal material, inoculate in 10 mL of LB+Ampicillin broth, mix, and incubate at 37 °C ± 1°C for 24 h± 1 h. 18 hours is also fine - note that it is best to treat all samples in the same way.

4h

2 Streak a SCAi medium agar plate

Streak to isolate single colonies, using a 10 µl loop. Use a Petri dish (90 mm is fine) of SCAi medium.

Incubate at 37°C ± 1°C for 48 h ± 1 h.

Sometimes, typical colonies (yellow, moist, dome-shaped) can be recognized after 24h culture on plates, but 48 h is much better to discriminate Klebsiella-looking colonies from other ones (E. coli colonies are typically white because they do not use inositol).

It can happen that the medium, which should initially be blue, turns completely yellow, when there are many inositol-fermenting colonies (typically Klebsiella). In these cases, discriminating yellow colonies is less easy. Diluting before streaking could help in these cases.

3 Purification of suspect K. pneumoniae colonies

Typical Klebsiella spp. colonies are yellow on SCAI medium.

Select suspect Klebsiella pneumoniae colonies and pick for subculture before bacterial identification.

If colonies are numerous and close to each other, re-isolate the colony on another SCAi agar plate to control for purity. Incubate for up to 48h.

4 Mixed-colony storage for future studies

If desired, after picking selected colonies, sweep the remaining SCAi plate content and freeze it at -80°C (e.g., for mixed colonies sequencing) using CryoBank tubes or equivalent (e.g., in house BHI + 15% glycerol medium).

2h

5 Identification

Streak the selected colonies onto the surface of a non-selective agar medium (e.g., LB or TSA) in a manner which will allow isolated colonies to develop.

Incubate plates at 37 °C ± 1°C for 24 h ± 1 h.

Determine species of purified suspect K. pneumoniae colonies using MALDI-TOF mass spectrometry and/or species-specific PCR.

6 Storage of bacterial strains

Freeze strains confirmed as Klebsiella pneumoniae (or its related species, which also grow on SCAi) at -80°C using CryoBank tubes or equivalent (e.g. BHI + 15% glycerol medium).

If several morphotypes are available, you may want to store one colony per morphotype.