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Isolation of Klebsiella strains from water samples

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

Klebsiella Research and Surveillance

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ABSTRACT

This protocol is intended for isolation of Klebsiella strains from water samples.
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 Buffered Peptone Water (BPW), and plating on SCAi (Simmons Citrate with Inositol) agar.
This protocol was optimized by the MedVetKlebs consortium.

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
myo-inositol	I5125-50 g	Sigma Aldrich
Simmons citrate	64834-500g	BioRad Sciences

MATERIALS TEXT

1. 0.45µm filters

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.

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%). Distribute 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 Buffer Peptone Water (BPW) solution and SCAi agar plates.

Pre-treatment of sample

1 Water samples are collected in 5L sterile containers and transported to the laboratory.
Six samples are collected at each site to give a total of 30 litres per sample.

The water samples are processed using the CapE system by placing the submersible pump into the pooled water samples.

NOTE: these first two steps might vary according to the each partner and systems available to collect water samples.

2 The 0.45µm filter is folded up using sterile forceps and placed in 100mL of **Buffered Peptone Water (BPW)** for **18-24** hours incubation at **42˚C ± 1°C**.

NOTE: Place all filters used per sample (30L) in the one 100mL BPW container.

Streak a SCAi medium agar plate

3 Following 18-24h incubation, using a 10µl loop, streak for single colonies onto the surface of a small petri dish (90 mm) of **SCAI medium** and incubate at **37°C ± 1°C** for **48h ± 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.

Purification and identification of suspect *Klebsiella* colonies

4 Typical *Klebsiella* spp. colonies are yellow on SCAI medium.

Select suspect *Klebsiella* colonies for subculture and bacterial 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.

NOTE: 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.

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

Mixed-colony storage for future studies (additional)

6 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).

Storage of bacterial strains

7 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.

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