

MedVetKlebs	WP1: <b><i>Klebsiella pneumoniae</i> from water samples</b>	Rev. 1 21 <sup>st</sup> March 2019
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## Procedure to isolate *K. pneumoniae* from water samples

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.
2. 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.

3. The 0.45µm filter is folded up using sterile forceps and placed in 100mL of **buffered peptone water** for 18-24 hours incubation at **42°C**. Place all filters used per sample (30L) in the one 100mL buffered peptone water container.
4. Following 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 **48 h ± 1 h**.

## Purification and Identification of suspect *K. pneumoniae* colonies

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

3. Select suspect *Klebsiella pneumoniae* colonies<sup>1</sup> (if there are several morphotypes, take one or two of each) from each plate for subculture and bacterial identification. **Sweep the remaining plate content and freeze it at -80°C (for mixed colonies sequencing)** using CryoBank tubes or equivalent (e.g. *in house* BHI + 15% glycerol medium).
4. Streak the selected colonies<sup>1</sup> onto the surface of a non-selective agar (e.g. LB or TSA) medium in a manner which will allow isolated colonies to develop. Incubate plates at 37 °C ± 1°C for 24 h ± 1 h.
5. Determine species ID in all purified suspect *K. pneumoniae* colonies using MALDI-TOF MS and/or qPCR ID phylogroups.
6. Freeze only isolates confirmed as *Klebsiella pneumoniae* complex at -80°C using CryoBank tubes or equivalent (e.g. *in house* BHI + 15% glycerol medium). Store only one isolate *per* morphotype.

<sup>1</sup>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.