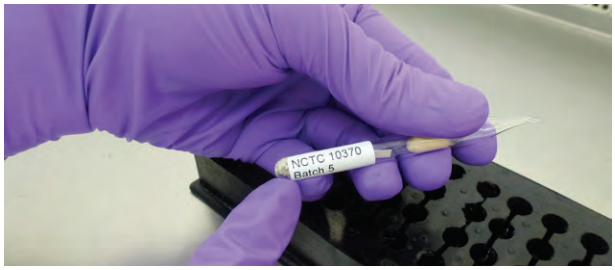
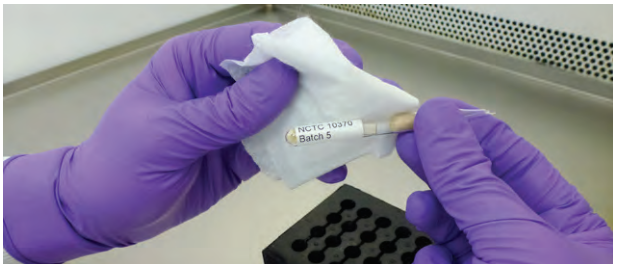
How to open and grow your freeze-dried NCTC strains

All NCTC bacterial cultures should be considered as potentially hazardous and should be opened by people who are trained in microbiological techniques and familiar with working in facilities with the containment requirements appropriate for the biosafety level of the cultures.

NCTC recommends that glass ampoules are opened in a biological safety cabinet designed to protect the worker against inhalation of aerosols (in the UK, this means a Class I, Class II or Class III cabinet). If that is not possible, wear personal protective equipment including gloves, safety goggles or visor, and protective clothing, and hold the ampoule away from your body when opening. A video of the following process is also available at: www.phe-culturecollections.org.uk/how-to-open-nctc-ampoules

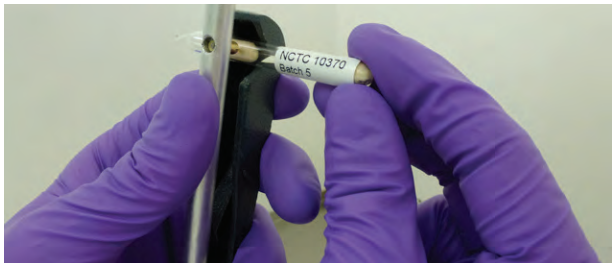
1. Prepare the appropriate medium necessary for reviving the strain and check the required incubation conditions.

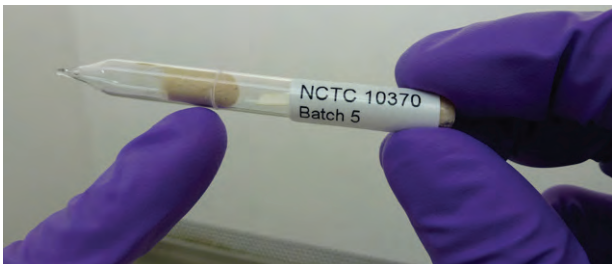




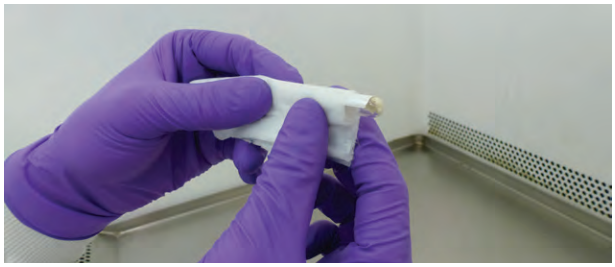
2. Identify the culture by the NCTC number on the paper inside or label on the ampoule.

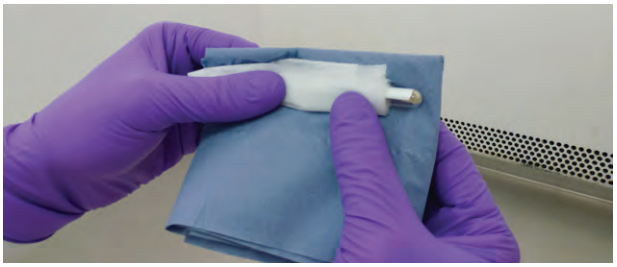
3. Clean the outside of the ampoule using a disinfectant wipe or tissue soaked in 70% alcohol.





4. Make a deep score around the circumference of the ampoule, using a diamond cutter, diamond pen or glass file halfway down the cotton wool plug. You can now either open the ampoule by 'wrapping and snapping' as shown in steps 5 – 8 or by using a heat source technique†.





5. Wrap the ampoule in a disinfectant wipe or a tissue soaked in 70% alcohol.

6. Then wrap with paper towels, tissue or gauze to act as a padding layer. This is to prevent accidental injury when the ampoule is snapped open.

† Using a heat source: As an alternative to the wrapping and snapping steps 5 – 8, heat a thin glass rod or pipette capillary (with a diameter of less than 3mm) until the tip is red hot and molten, then quickly and firmly apply the heated end to the scored mark on the ampoule to crack it around the entire circumference. If the first attempt is unsuccessful, repeat using another piece of heated glass. Ensure that the glass rod/capillary being used is heated adequately and applied quickly (before it cools) to the score mark on the ampoule. Then continue from step 9.

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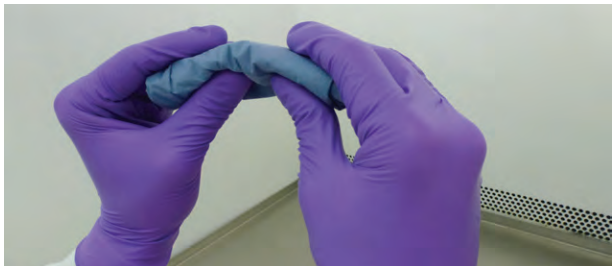
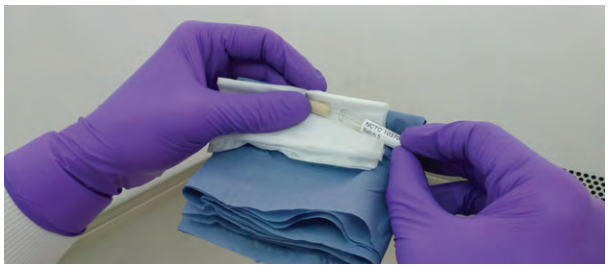
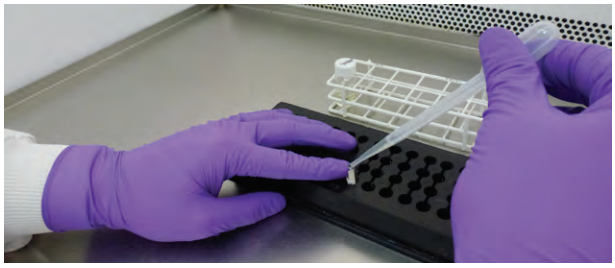


7. Snap the ampoule while wrapped where the score mark was made.

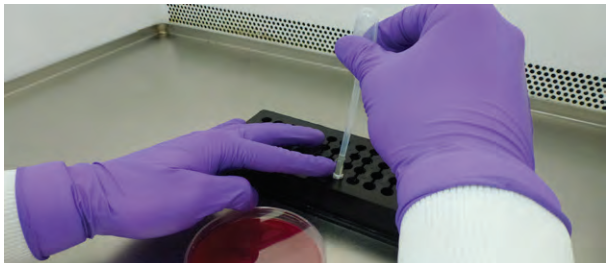


8. Carefully unwrap the ampoule as fragments of glass may be present in the tissue. Discard the tissue and the ampoule tip into a sharps bin.

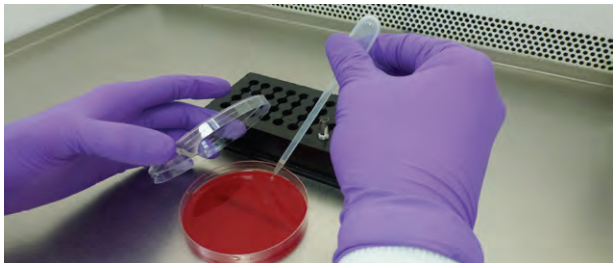
9. Once opened, air will enter the ampoule because the vacuum is no longer intact. This air will be filtered by the cotton wool plug which may have been in contact with dried bacterial culture so must be discarded safely. Remove the plug with forceps if the plug does not come away with the tip of the ampoule before reconstituting the culture.



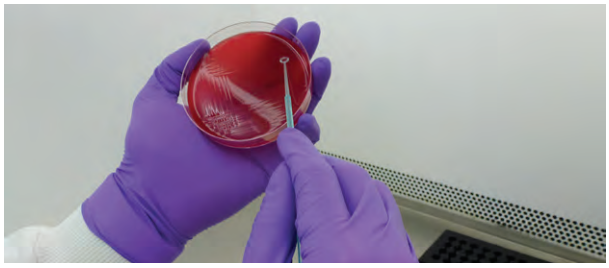
10. To reconstitute, transfer approximately 0.5ml of broth, when necessary enriched with blood, to the ampoule.



11. Allow the microorganisms to rehydrate for 5-10 minutes. Mix very carefully to avoid creating aerosols or causing the contents to froth. Check all material is dissolved.



12. Subculture onto appropriate culture media, ideally including a solid medium to make it easier to detect any contaminants that may have been introduced as the ampoule was opened. Discard the used ampoule into a sharps bin.



13. Most freeze-dried bacteria will grow within a few days although some may require a slightly extended incubation period than normal because a proportion of the bacteria will be sub-lethally injured due to the preservation process and will need time to recover on the nutrient-rich medium.