CryoCare Bacterial Preservation

INTENDED USE:
CryoCare is a method of preserving bacterial and other cultures for long periods avoiding freeze drying or daily culture transfers. CryoCare is a vial containing chemically treated porous beads in a cryopreservative fluid of TSB + glycerol with a hypertonic additive. Each bead serves as a carrier for the culture during storage.

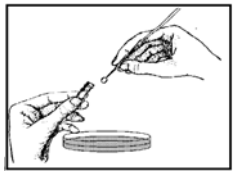
PROCEDURE FOR PREPARATION:
1. Remove the cap, being careful not to contaminate the contents. Inoculate the CryoCare vial with young (18-24 hours) colonial growth of a pure culture of the organism being preserved to approximate a McFarland 3-4 standard, using a sterile loop. (optional method) Use a sterile pipette to harvest and emulsify the colonies into the CryoCare vial by using a squeezing action. The same pipette may then be used to extract the excess fluid. (step 3)Liquid cultures can be lightly centrifuged and the deposit used as above. (figure 1)
2. Cap the tube and invert 6 times. DO NOT VORTEX. (figure 2) If using no beads, cooked meat, or skim milk, go to step 4.
3. Let vial stand for 30 seconds. The organism will now be bound to the beads. Remove the excess cryopreservative fluid leaving the beads as free of liquid as possible. (figure 3) A sterile pipette is best for this procedure. Close the vial finger tight.
4. Record the culture details on the vial and store at minus 70ºC. (see PRECAUTIONS, paragraph 3)

PROCEDURE FOR USE - RECOVERY:
1. Remove the vial from the freezer.
2. If using vials without beads, scrape a little liquid from the top of the vial with a sterile loop or scraper then streak as in figure 6. Return the vial to the freezer quickly.
3. If using beads, carefully open the vial and remove a single bead with a sterile needle, forceps, or the special CryoCare hook available from KEY Scientific. (figure 5)
4. Rub the bead over solid medium (figure 6), streak from point of contact, or drop into appropriate growth broth. Some organisms perform better using the broth method. Beads should not be returned to the vial after removal.

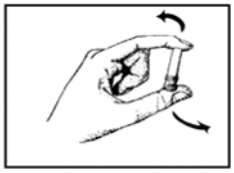
NOTE: For COOKED MEAT, SKIM MILK, and NO BEAD CRYOCARE scrape liquid from the unthawed tube to use for streaking the plate. Do not defrost before using.

STORAGE PRIOR TO USE:
Unused CryoCare may be stored at room temperature up to 30ºC. Shelf life of frozen organisms is dependent upon freezer temperature and frequency of use and must be determined by each user by periodical testing. (see notation under PRECAUTIONS, paragraph 3.)

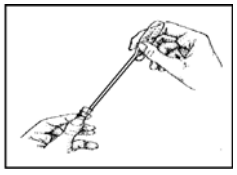
1. Inoculate



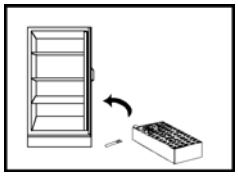
2. Rotate



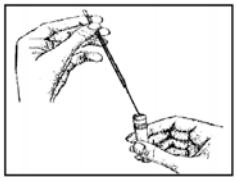
3. Remove the cyropreservative.



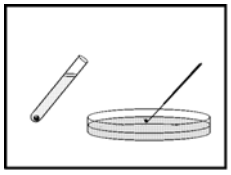
4. Freeze (-70º C is best.)



5. Remove a bead.



6. Inoculate media.



PRECAUTIONS AND LIMITATIONS:
1. CryoCare provides a method of extending storage of microorganisms and is intended for laboratory use only. Aseptic procedures should be observed at all times to ensure the integrity of the organism and for safety of the microbiologist. Observe biohazard precautions when disposing of used vials.
2. When removing a bead for recovery, precautions should be taken to avoid thawing of the remaining beads. An acrylic cryoblock is available for this purpose. Freezing the cryoblock and placing the vial into it while out of the freezer extends the life of the organism. The cryoblock may also be used for quick freezing fastidious organisms by keeping it frozen and placing the inoculated vial into it immediately upon setup.
3. Users have reported successful recovery up to 2 years from setup when storing at warmer temperatures (as warm as minus 10ºC) although minus 70ºC is the desired temperature. (see STORAGE)
4. CryoCare™ should not be used if any of the following conditions are present:
   a) turbidity in the vial before inoculation which indicates possible contamination.
   b) before use, any evidence of leakage (loss of cryopreservative).
5. CryoCare™ is supplied in a variety of colors to provide a method of color-coding. The colors contain the same medium and are not different in performance characteristics.
6. CryoCare™ is non-hazardous. Discard spills of uninoculated product as normal waste. Spills of inoculated product should be treated as biohazard material. CryoCare™ is sold in boxes of 50 in reusable, autoclavable polypropylene containers as described below. Containers may also be purchased separately.

CATALOG NUMBERS

KS70MI50	MIXED EACH COLOR
KS70YE50	YELLOW
KS70GR50	GREEN
KS70RE50	RED
KS70WH50	WHITE
KS70BL50	BLUE
KS70X	NO BEADS
KS70G	BEADS ONLY
KSMB50	SKIM MILK BASE
KSCM50	COOKED MEAT NO BEADS
KS80RE50	BRUCELLA BASE RED BEADS
KS80BL50	BRUCELLA BASE BLUE BEADS

CryoCare HOOK-KSC4010- Sterilizable bent hook to remove the frozen bead. The strong shaft makes it easy to separate the beads.
CryoSafe- **KSC18847**- 12 well cold box keeps vials frozen 1 ½ hours at -15C when taken from -20C freezer. May also be used for quick freezing on the counter.

REFERENCES
1. Medical Laboratory Sciences, 1985,42:289-290
2. Feltham et al, Journal Applied Bacteriology, 1978, 44:313-316
3. Nagel, Janice G. and Cunz, Applied Microbiology, 1971, 23:837-838
4. White & Sand RL, Medical Laboratory Sciences, 1985, 42:289-290 (UK)
5. Park, Choong H.,Am. J. of Clin. Pathology, 1976, Vol. 66,

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