Microbank™

Worldwide Performance Portfolio

Pro-Lab Diagnostics Canada
20 Mural Street, Unit #4
Richmond Hill, ON L4B 1K3
Canada
Toll Free: 1-800-268-2341
Tel: (905) 731-0300
Fax: (905) 731-0206
support@pro-lab.com

Pro-Lab Diagnostics U.K.
7 Westwood Court
Neston, Cheshire
CH64 3UJ, United Kingdom
Tel: 0151 353 1613
Fax: 0151 353 1614
uksupport@pro-lab.com

Pro-Lab Diagnostics U.S.A.
21 Cypress Blvd., Suite 1070
Round Rock, TX, 78665-1034
U.S.A.
Toll Free: 1-800-522-7740
Tel: (512) 832-9145
Fax: 1-800-332-0450
ussupport@pro-lab.com

Contents

Microbank™ Worldwide Performance Portfolio

A Selection of References for Microbank™

1. **An Internal Quality Assessment Scheme for Clinical Bacteriology Microbank™.**
   Peter Taft, Bury District General Hospital. Pro-Lab Pulse Article

2. **Public Health Laboratory Anaerobic Reference Centre, Cardiff UK.**
   Dr J Brazier, Dr V Hall

3. **Storage and Repeated Recovery of Neisseria Gonorrhoeae**
   A Moyes and H Young, Scottish Gonococcal Reference Library

4. **Long Term Storage of Fastidious Camplylobacter and Helicobacter.**
   K Illingworth, E Le Roux, A J Lastovica,
   Medical Microbiology, Red Cross Children's Hospital, Cape Town, South Africa

5. **Recorded Storage of Salmonella spp, at the Salmonella Reference Laboratory, Veterinary Laboratory Agency, UK**

6. **Cryopreservation of Fungal Spores**
   D Chandler, Horticultural Research Centre, Warwick, UK

7. **Storage of NCTC Organisms Selected for Quality Control and Laboratory Accreditation Requirements**
   T Donovan, PHLS, UK.

8. **Validation of Microbank™ for Storage of Brucella spp**
   J Tucker, L Perret, Statutory and Exotic Bacteria Department, VLA, UK

9. **Long Term Preservation of Fungal Isolates in Commercially Prepared Cryogenic Microbank™ Vials**
   A Espinal-Ingroff, D Montero, E Martin-Mazuelos
   VCU Medical Centre, Virginia USA and Valme University Hospital, Spain

MICROBANK™

The original unique system with proven documented Performance references

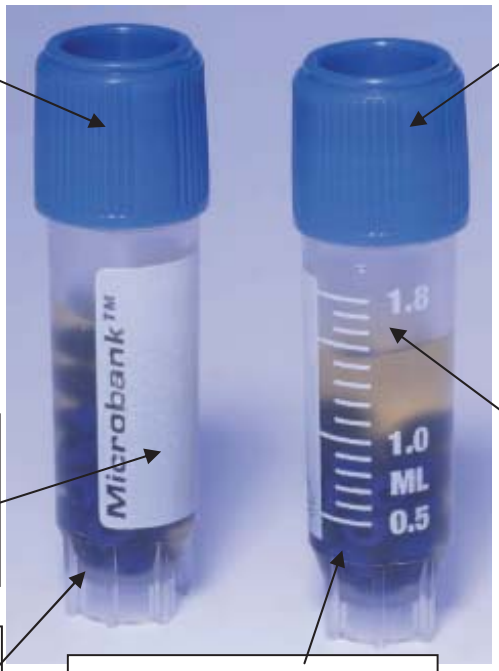
Microbank™ - The Original Long Term

Bacterial & Fungal Storage System

Microbank™ is a convenient, ready-to-use system designed to greatly simplify the storage and retrieval of bacterial cultures. It comprises of a unique cryovials system incorporating treated beads and a special cryopreservative solution.

Microbank™ has proven performance and is now the natural choice for Microbiologists worldwide including many specific reference culture collection centres. Microbank™ is a more reliable method for maintaining important cultures than repetitive subculture, which can result in altered characteristics, lost organisms, or contaminated cultures. Microbank™ provides microbiologists with a much simpler option to traditional methods of lyophilization or use of glycerol broth.

Large 2 ml size vials with triple depth external threaded cap. The larger cap reduces the possibility of contamination and the wider tube diameter provides more room for mixing to ensure beads are properly coated.



Available in five colours which provides laboratories a system to colour code different bacterial species.

Larger writing area allows for complete coding and reference data.

Specially formulated preservative ensures longer survival of fastidious bacteria and higher quantitative recoveries.

Industry standard robust cryovial which can withstand snap freezing with liquid nitrogen.

Chemically treated beads provide improved bacterial adhesion.

Microbank™
Product Availability.

Advanced presentation of 80 vials supplied in a plastic freezer box manufactured from durable plastic with “see through” lids, number locator printed screens and tube collection device.

PL.170/B	Microbank™ - Blue Colour Beads & Cap	80 vials
PL.170/G	Microbank™ - Green Colour Beads & Cap	80 vials
PL.170/R	Microbank™ - Red Colour Beads & Cap	80 vials
PL.170/Y	Microbank™ - Yellow Colour Beads & Cap	80 vials
PL.170/LB	Microbank™ - Light Blue Colour Beads & Cap	80 vials
PL.170/M	Microbank™ - Mixed (16 Vials Each Colour)	80 vials

Dry Microbank™

Supplied in the same format as traditional Microbank™ without the specially formulated cryopreservation solution.

PL.172/B	Microbank™ Dry - Blue Colour Beads & Cap	80 vials
PL.172/G	Microbank™ Dry - Green Colour Beads & Cap	80 vials
PL.172/R	Microbank™ Dry - Red Colour Beads & Cap	80 vials
PL.172/Y	Microbank™ Dry - Yellow Colour Beads & Cap	80 vials
PL.172/LB	Microbank™ Dry - Light Blue Colour Bead & Cap	80 vials
PL.172/M	Microbank™ Dry - Mixed (16 Vials Each Colour)	80 vials

Microbank™ - Accessories

PL.155-1	Cryoblock (Insulated Aluminium Block)	20 Well
PL.156	Cryoblock - Replacement Insulated Base & Lid	4 Pack
PL.165	Microbank™ Reference Cards	12 Cards
PL.166	Aluminum Cryocanes	12 Canes
PL.169/B	Microbank™ Freezer Storage Box – Blue	24 Pack
PL.169/R	Microbank™ Freezer Storage Box – Red	24 Pack
PL.169/B-1	Microbank™ Freezer Storage Box – Blue	Each
PL.169/R-1	Microbank™ Freezer Storage Box – Red	Each

PRODUCT CODE PL.170

Microbank™
Instructions for Use

INTENDED USE

Microbank™ is a sterile vial containing porous beads which serve as carriers to support microorganisms.

SUMMARY AND EXPLANATION

Long term storage of microorganisms is a challenge in routine microbiology. Organisms should be stored at low temperatures utilizing a mechanical technique that offers the least possibility of disturbance, yet, permits ready access to stored material. Microbank™ offers a solution to this problem.

DESCRIPTION

Individual coloured beads are packaged approximately 25 beads in a cryovial containing cryopreservative. The beads are washed and are of a porous nature allowing microorganisms to readily adhere onto the bead surface. After inoculation the cryovials are kept at -70°C for extended storage. When a fresh culture is required, a single bead is easily removed from the vial and used to directly inoculate a suitable bacteriological medium.

PROCEDURE

A. PREPARATION

1. Using a permanent marker, code the vial as desired, one organism per vial to be inoculated. (See also step 6).

2. Under aseptic conditions open the screw cap cryovial.

3. Inoculate the cryopreservative fluid with young colonial growth (18-24 hours) picked from a pure culture to approximately a 3-4 McFarland standard.

4. Close vial tightly and invert 4-5 times to emulsify organism. **DO NOT VORTEX**!

5. At this point the microorganisms will be bound to the porous beads. The excess cryopreservative should be well aspirated leaving the inoculated beads as free of liquid as possible. Close the vial finger tight.

6. Record the inoculation coding on the grid provided or on other permanent record as desired.

7. Store the inoculated cryovial at -70°C for best long term results.

B. RECOVERY

1. Under aseptic conditions, open the cryovial and using a sterile needle or forceps remove one coloured bead. Close the vial finger tight and return as soon as possible to low temperature storage. Excessive changes in temperature reduce the viability of the organisms.

2. The inoculated bead may then be used to directly streak on to solid medium or may be dropped into an appropriate liquid medium.

3. When used as recommended, each cryovial will store approximately 25 identical potential cultures.

LIMITATIONS

1. Microbank™ is offered solely as a means of providing extended storage possibilities for organisms.

2. In use, aseptic technique should be practiced to ensure continued integrity of the stored microorganism.

3. Microbank™ should not be used if any of the following conditions are present before inoculation:

   (a) the vial shows any evidence of leakage (loss of cryopreservative)

   (b) turbidity in cryopreservative suggesting contamination

   (c) the expiry date on the outer label has elapsed

4. After removal, beads should not be returned to the cryovial for any reason.

5. Microbank™ is supplied in a variety of colours. These colours do not imply any change in the product function. They are provided only for colour coding convenience.

SAFETY PRECAUTIONS

1. A microbiological safety cabinet should be used when making and manipulating a heavy suspension of a culture.

2. Observe biohazard precautions when discarding used or partly used cryovials.

3. When storing Microbank™ in liquid nitrogen the following precautions should be taken:

   (a) ensure that the cryovial screw cap is tightened normally: over-tightening may cause distortion of the silicone O-ring in the cap which may cause leakage

   (b) ensure that the thread of the cryovial and screw cap is completely dry before closing: liquid drops will impair the seal in liquid nitrogen

   (c) all Microbank™ vials should always be stored in the gas phase, above liquid nitrogen. If immersed, they might develop leaks or even shatter when returned to room temperature

   (d) when removing vials from liquid nitrogen containers always use safety equipment such as gloves, hoods, face shields etc…..

PRESENTATION Microbank™ is packaged in shelf packs of 80 vials.

STORAGE

Before use, unused Microbank™ may be stored at 4°C or at room temperature but kept away from direct light. Stored under these conditions Microbank™ may be used up to the date of expiry shown on the product label.

REFERENCES

1. *White and Sand, R.L. 1985. Medical Laboratory Sciences **42**:289-290(U.K).*
2. *Feltham et al. 1978. Journal of Applied Bacteriology. **44**:313-316.*
3. *Nagel, J.G. and Cunz, L.J. 1971. Applied Microbiology, **23(4)**:837-838*

Microbank™
Preservation & Retrieval Procedure

1. Inoculate the cryopreservative with colonial growth (18-24 hours) from pure culture.

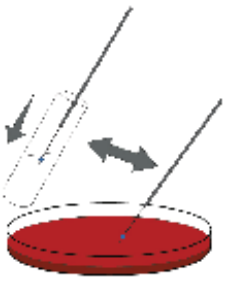
2. Close Vial tightly and invert 4-5 times to emulsify organism. Do not vortex.

3. Aspirate excess cryopreservative using sterile pipette.

4. Store inoculated cryovial in appropriate freezer or liquid nitrogen.

5. Recover inoculated beads under aseptic conditions using a sterile needle or forceps.

6. The bead may then be used to inoculate appropriate solid or liquid media.



Reference

An Internal Quality Assessment Scheme for Clinical Bacteriology using Microbank™
Peter Taft. Microbiology. Royal Oldham Hospital

The development of an internal quality control scheme in clinical bacteriology has been hampered by a lack of suitable cultures. However, work undertaken using Mircrobank™ beads may provide a solution to this problem.

Internal Quality Assessment in Clinical Bacteriology

One definition of quality involves meeting the predetermined requirements of users of a product or service. An effective e quality management system (QMS) determines the needs and expectations of users and evaluates the processes, responsibilities and resources required to meet quality objectives. In the laboratory, quality control (QC) procedures should be used in conjunction with external and internal quality assessment (QA), audit and equipment monitoring as an integral part of the QMS. Quality control permits the day to day monitoring of assay, operator and equipment performance. It should detect both random and systematic errors.

Criteria for Quality Control Material

In general, QC material should be independent of kit controls, be stable over a long period of time, be of sufficient volume to monitor within and between kit and reagent and batches, and give results within a clinically significant range (for bacteriological cultures, this means target organisms).

Unfortunately, availability of suitable QC material presents a problem in developing a suitable internal QA scheme in clinical bacteriology. One scheme established for QA in clinical bacteriology involves the submission of anonymised original specimen for analysis[1]; however, problems associated with this type of QC material include:

- Failure to meet at least two of the criteria mentioned above
- Repeat inoculation of a swab on a second set of plates may present a different picture (depending on the number of organisms originally present)
- A high percentage of bacteriology samples are negative and this does not challenge the ability to isolate ‘target’ organisms
- Although this type of scheme assesses reproducibility, it does not detect systematic errors (because you don’t know what you might be missing)

Microbank™ Beads

An alternative scheme using simulated specimens preserved on Microbank™ beads (Pro-Lab Diagnostics) was evaluated over a six-month period by Peter Taft, Microbiologist at the Royal Oldham Hospital. Microbank™ offers a ready-to-use designed to simplify the storage and retrieval of bacterial and fungal cultures.

Comprising a special cryovial system that incorporates treated beads and a cryopreservative solution, Microbank™ provides a more reliable means of maintaining cultures than is possible with repetitive subculture, which can result in contaminated cultures, lost organisms or changed characteristics. The special formulated preservative ensures longer survival of fastidious organisms and higher quantitative recoveries. This makes the Microbank™ system ideal for QC applications where organism integrity, consistency and quality are of paramount importance.

Each 2 ml Microbank™ vial contains approximately 25 beads, providing the facility for repeat culture of the original organism using a simple procedure. Extensive, proven performance reference data, updated for 2005, is available on request from Pro-Lab Diagnostics.

To demonstrate the utility of the Microbank™ system, seven simulated specimens were prepared. Briefly, using freshly isolated colonies, a suspension of the target organism was prepared (equivalent to a McFarland 2 standard) in a Microbank™ vial. To simulate a clinical specimen, colonies of typical mixed normal flora were added to make the final suspension equivalent to a McFarland 5 standard **(Table 1).** The vial was mixed thoroughly and the contents were decanted into a Petri dish. Using sterile forceps, each bead was placed in an individual cryotube, which was then labelled and stored frozen at -80°C.

Table 1 – Simulated Specimens

No	Specimen Type	Target Organism(s)	Other Organisms
1	Throat Swab	Group A streptococcus	Mixed oral flora
2	Throat Swab	*Corynebacterium diphtheriae*	Mixed oral flora
3	Sputum	*Haemophilus influenzae*	Mixed oral flora
4	Sputum	*Streptococcus pneumoniae*	Mixed oral flora
5	Wound Swab (burn)	*Staphylococcus aureus* *Pseudomonas aeruginosa*	Coagulase-negative staphylococcus
6	Faeces	Group D salmonella	*Escherichia coli* *Proteus mirabilis*
7	Faeces	*Escherichia 0157*	*Escherichia coli (sorbitol-positive*

Day-to-Day Use

Each of the seven simulated specimens was processed once a week. Briefly, a vial was removed from the -80°C freezer and allowed to warm to room temperature. Nutrient broth (1ml) was added to the bead and mixed thoroughly. A routine set of culture plates was inoculated from a swab dipped in the broth. Finally, specimen details were entered on the laboratory computer system, following an agreed format.

Culture Results

Over a six-month period (June to December 2003) all target organisms were isolated and correctly identified from six out of the seven simulated specimens. On two occasions, isolation of *Haemophilus influenzae* from specimen 3 failed.

Overview

Once prepared, simulated samples are simple to set up, record and score, and are inexpensive to prepare. In addition, they satisfy all the criteria for a QC material. Drawbacks include the fact that simulated samples are not 'real' specimens, and that some organisms may survive better than others at -80°C after 'pooling'. Fastidious organisms such as *Neisseria gonorrhoeae, Campylobacter* spp. and anaerobic organisms have yet to be tested using the Microbank™ system.

It may be argued that staff would soon get to know which organisms are present in the samples, but this argument also can be applied to most QC material used across pathology and is not relevant unless a 'blame culture' exists in the

organisation. Used properly, however, a successful internal QC scheme will increase confidence in results and, in conjunction with external QA and audit, identify problems and assess the effectiveness of remedial measures.

Reference

[1]Constantine CE, Amphlet M, Farrington M, et al. Development of an Internal Quality Assessment Scheme in a Clinical Bacteriology Laboratory.
J Clin Pathol 1993; 46: 1046-1050

Reference

Microbank™ Storage (10 years) Trial for Anaerobes

Method and Materials

Microbank™ vials were inoculated with clinical isolates of obligatory anaerobic bacteria referred to the PHLS Anaerobe Reference Unit (now the National Public Health Service for Wales Anaerobe Reference Laboratory, ARL) for confirmation of identity, and were frozen at -80°C for ten years. Isolates for study (n = 100) were selected to represent the range of species commonly isolated from clinical material. One bead from each vial had been removed to demonstrate viability after five years and seven years. Beads may have been removed on other occasions.

One bead from each vial was placed aseptically onto Anaerobe Basal Agar (Oxoid, Basingstoke) containing 5% horse blood. Plates were spread for single colonies and were incubated promptly at 37°C in an anaerobic chamber (Concept Plus, Biotrace Fred Baker, Runcorn) for 48 hours.

On removal from the chamber, plates were examined for growth of colonies characteristic of the intended isolates. If growth was not apparent at this stage, plates were re-incubated for a further three days.

Results

All of 100 strains of anaerobic bacteria of clinical origin were viable after storage in Microbank™ vials at -80°C for ten years. Most strains yielded moderate to heavy growth from a single bead after 48 hrs incubation but three strains yielded only light growth after five days incubation. These comprised one of the two strains of *Prevotella denticola* examined, one of four *Fusobacterium nucleatum* strains and one strain of *Fusobacterium varium.*

Discussion and Conclusions

One hundred anaerobic bacteria representing a wide range of genera isolated from clinical sources remained viable after ten years storage in Microbank™ vials at -80°C. The same 100 vials were previously sampled for viability after five and seven year's storage. However, 34 of those 100 isolates have changed names since their original identification at the **ARL.** These changes reflect advances in taxonomy and identification methods over the decade.

Some species have simply been placed in novel genera and, in some cases, have changed gender in the process e.g. *Eubacterium lentum* became *Eggerthella lenta.* The gram-positive anaerobic cocci have undergone major taxonomic review, resulting in the removal of most former *Peptostreptococcus* species to novel genera e.g. *Finegoldia, Anaerococcus, Peptoniphilus.* Additionally, several novel species of anaerobic cocci have been described e.g. *Peptoniphilius harei, Peptoniphilus ivorii.* Several novel species have been described in other genera e.g. *Prevotella, Porphyromonas, Actinomyces.* The identification methods used at the **ARL** have been revised to accommodate such taxonomic changes.

The **ARL** has developed novel molecular methods, principally amplified 16S ribosomal DNA restriction analysis **(ARDRA)** for identification of *Bacteroides, Prevotella* and *Porphyromonas* and for *Actinomyces* and other non-sporing gram-positive bacilli. **ARDRA** is more accurate and discriminatory than conventional phenotypic tests for identification of these groups; consequently some strains examined in the Microbank™ storage trial have been re-designated as a result of retrospective identification by **ARDRA**. Application of **ARDRA** to isolates stored at the **ARL** has resulted in the recognition of several novel *Actinomyces* species e.g. *Actinomyces cardiffensis* and a novel genus and species *Varibaculum cambriense.* By chance, two strains previously included in the Microbank™ storage trial as *Actinomyces* species have subsequently been identified as members of these novel species.

Conclusion

Microbank™ vials are easy to use, compact, maintain viability and, therefore, are convenient for the long-term storage of anaerobic bacteria. The vials are particularly practical for repeated retrieval of strains as they ensure that the same strain is sub-cultured on each occasion with minimal effort. The **ARL** holds a collection of approximately 20,000 referred isolates dating back to the early 1980's. Isolates received in the past decade have been stored in Microbank™ vials. This collection is a valuable resource for retrospective research in fields such as evaluation of novel identification methods, monitoring of antimicrobial susceptibilities and development of molecular typing schemes.

Dr. Val Hall, Anaerobe Reference Laboratory, NPHS Microbiology Cardiff, University Hospital of Wales, Cardiff CF14 4XW

MICROBANK™ STORAGE 10 YEAR TRIAL					
ARL ref.	Organism		Growth after		
	Previous Identification	Current Identification	5yrs	7yrs	10yrs
R5555	*Clostridium perfringens*	*C.perfringens*	+	+	+
R5558	*C.paraputrificum*	*C.paraputrificum*	+	+	+
R5559	*C.butricum/beijerinckii*	*C.butricum/beijerinckii*	+	+	+
R5560	*C.septicum*	*C.septicum*	+	+	+
R5572	*C.tetani*	*C.tetani*	+	+	+
R5573	*C.difficile*	*C.difficile*	+	+	+
R5584	*C.clostridioforme*	*C.clostridioforme*	+	+	+
R5586	*C.perfringens*	*C.perfringens*	+	+	+
R5601	*C.bifermentans*	*C.bifermentans*	+	+	+
R5606	*C.novyi type A*	*C.novyi type A*	+	+	+
R5628	*C.ramosum*	*C.ramosum*	+	+	+
R5635	*C.cadaveris*	*C.cadaveris*	+	+	+
R5642	*C.sordellii*	*C.sordellii*	+	+	+
R5759	*C.sporogenes*	*C.sporogenes*	+	+	+
R5760	*C. glycolicum*	*C. glycolicum*	+	+	+
R5570	*bacteroides thetaiotaomicron*	*B.thetaiotaomicron*	+	+	+
R5587	*B.fragilis*	*B.fragilis*	+	+	+
R5589	*B.fragilis metronidazole resist.*	*B.fragilis metronidazole resist.*	+	+	+
R5600	*B.distasonis*	*B.distasonis*	+	+	+
R5620	*B.fragilis*	*B.fragilis*	+	+	+
R5631	*B.uniformis*	*B.uniformis*	+	+	+
R5745	*B.ovatus*	*B.ovatus*	+	+	+
R5755	*B.thetaiotaomicron*	*B.thetaiotaomicron*	+	+	+

ARL ref.	Organism		Growth after		
	Previous Identification	Current Identification	5yrs	7yrs	10yrs
R5762	*B.distasonis*	*B. distasonis*	+	+	+
R5791	*B. vulgaris*	*Bacteroides species*	+	+	+
R5801	*B. ovatus*	*B. thetaiotaomicron*	+	+	+
R5867	*B. splanchnicus*	*B. splanchnicus*	+	+	+
R5868	*B. splanchnicus*	*B. splanchnicus*	+	+	+
R5933	*B. thetaiotaomicron*	*B. thetaiotaomicron*	+	+	+
R5956	*B. fragilis*	*B. fragilis*	+	+	+
R5783	*Prevotella loescheii*	*Prevotella species*	+	+	+
R5836	*Prevotella species*	*Prevotella species*	+	+	+
R5839	*Prevotella species*	*Prevotella species*	+	+	+
R5954	*Prev. denticola*	*Prev. denticola*	+	+	+
R5955	*Prev. melaninogenica*	*Prev. melaninogenica*	+	+/-	+
R5974	*Prev. melaninogenica*	*Prev. denticola*	+	+/-	+/-
R6080	*Prev. oris*	*Prev. oris*	+	+	+
R5550	*Porphyromonas asaccharolytica*	*Porph. Asaccharolytica*	+	+	+
R5807	*Porph. Endodontalis*	*Porph. Uenonis*	+	+	+
R5838	*Porphyromonas species*	*Porph. Levii*	+	+	+
R5995	*Porph. Endodontalis*	*Porph. Endodontalis.*	+	+	+
R6079	*Porph. Levii*	*Porphyromonas species*	+	+	+
R5565	*Fusobacterium necrophorum*	*Fusobacterium necrophorum*	+	+	+
R5585	*F. necrophorum*	*F. necrophorum*	+	+	+
R5641	*F. varium*	*F. varium*	+	+	+/-
R5716	*F. russii*	*F. russii*	+	+	+
R5748	*F. nucleatum*	*F. nucleatum*	+	+	+

ARL ref.	Organism		Growth after		
	Previous Identification	Current Identification	5yrs	7yrs	10yrs
R5769	*F. naviforme*	*F. naviforme*	+	+	+
R5778	*F. necrophorum*	*F. necrophorum*	+	+	+
R6000	*F. nucleatum*	*F. nucleatum*	+	+	+
R6003	*F. nucleatum*	*F. nucleatum*	+	+	+
R6066	*F. nucleatum*	*F. nucleatum*	+	+/-	+/-
R5562	*Peptostreptococcus asaccharolyticus*	*Peptoniphilus harei*	+	+	+
R5563	*Peptostreptococcus species*	*Peptoniphilus lacrimalis*	+	+	+
R5622	*Peptostreptococcus species*	*Peptostreptococcus species*	+	+	+
R5624	*Peptostreptococcus species*	*Anaerococcus octavius*	+	+	+
R5630	*Peptostreptococcus productus*	*Ruminococcus productus*	+	+	+
R5668	*Staphylococcus saccharolyticus*	*Staphylococcus saccharolyticus*	+	+	+
R5720	*Peptostreptococcus magnus*	*Finegoldia magna*	+	+	+
R5761	*Peptostreptococcus productus*	*Ruminococcus productus*	+	+	+
R5767	*Peptostreptococcus magnus*	*Finegoldia magna*	+	+	+
R5805	*Peptostreptococcus micros*	*Peptostreptococcus micros*	+	+	+
R5806	*Peptostreptococcus anaerobius*	*Peptostrep. Anaerobius*	+	+	+
R5826	*Streptococcus mutans*	*Strept. Mutans*	+	+	+
R5840	*Peptostreptococcus anaerobius*	*Peptoniphilus ivorii*	+	+	+
R5927	*Peptostreptococcus asaccharolyticus*	*Peptoniphilus harei*	+	+	+
R5997	*Peptostreptococcus micros*	*Peptostreptococcus micros*	+	+	+
R5569	*Eubacterium aerofaciens*	*Eubacterium aerofaciens*	+	+	+
R5598	*Eubacterium lentum*	*Eggerthella lenta*	+	+	+
R5670	*Eu. Lentum*	*Eggerthella lenta*	+	+	+
R5800	*Eubacterium aerofaciens*	*Eubacterium species*	+	+	+

ARL ref.	Organism		Growth after		
	Previous Identification	Current Identification	5yrs	7yrs	10yrs
R5837	*Eu. Lentum*	*Eggerthella lenta*	+	+	+
R5552	*Actinomyces naeslundii*	*A. naeslundii*	+	+	+
R5554	*A. israelii*	*A. naeslundii*	+	+	+
R5557	*A. israelii*	*A. gerencseriae*	+	+	+
R5568	*A. odontolyticus*	*A. odontolyticus*	+	+	+
R5571	*Actinomyces species*	*Actinomyces species*	+	+	+
R5619	*Actinomyces species*	*Varibaculum cambriense*	+	+	+
R5634	*A. naeslundii*	*A. naeslundii*	+	+	+
R5639	*A. turicensis*	*A. turicensis*	+	+	+
R5718	*Actinomyces viscosis*	*A. naeslundii*	+	+	+
R5774	*A. gerencseriae*	*A. gerencseriae*	+	+	+
R5556	*Bifodobacterium species*	*Bif. Longum*	+	+	+
R5588	*Bifodobacterium species*	*Bif. Longum*	+	+	+
R5824	*Bif. Longum*	*Bif. Longum*	+	+	+
R5921	*Bif. Animalis group*	*Lactobacillus catenaforme*	+	+	+
R5561	*Propionibacterium acnes*	*Prop. Acnes*	+	+	+
R5567	*Prop. Propionicum*	*Prop. Propionicum*	+	+	+
R5671	*Prop. granulosum*	*Prop. Avidum*	+	+	+
R5764	*Prop. Acne*	*Prop. Acnes*	+	+	+
R6085	*Lactobacillus acidophilus*	*L. acidophilus*	+	+	+
R6097	*L. acidophilus*	*L. acidophilus*	+	+	+
R5551	*Wolinella recta*	*Campylobacter gracillis*	+	+	+
R5669	*Camp. gracillis*	*Camp. Gracillis*	+	+	+
R5738	*Wolinella species*	*Camp. Rectus*	+	+	+

ARL ref.	Organism		Growth after		
	Previous Identification	Current Identification	5yrs	7yrs	10yrs
R5756	*Bacteroides ureolylicus*	*B.ureolyticus*	+	+	+
R6001	*B.ureolyticus*	*B.ureolyticus*	+	+	+
R6043	*Bilophilia wadsworthia*	*Bil. Wadsworthia*	+	+	+
R5675	*Veillonella parvula*	*Veill. Parvula*	+	+	+
R5848	*V.parvula*	*Veill. Parvula*	+	+	+

Reference
Microbank™ Storage (7 years) Trial for Anaerobes

Method and Materials

Microbank™ vials were inoculated with clinical isolates of obligately anaerobic bacteria referred to the PHLS Anaerobe Reference Unit for confirmation of identity, and were frozen at -80°C for seven years. Isolates for study (n = 100) were selected to represent the range of species commonly isolated from clinical material. One bead from each vial had been removed for culture after five years. Beads may have been removed on other occasions.

One bead from each vial was placed aseptically onto Fastidious Anaerobe agar (IDG, Bury) containing 5% horse blood. Plates were spread for single colonies and promptly incubated in an anaerobic chamber (Concept Plus, Fred Baker Scientific, Runcorn) for 48 hours.

On removal from the chamber, plates were examined for growth of colonies characteristic of the intended isolates. If growth was not apparent at this stage, plates would be re-incubated for a further three days but, in the event, this was not necessary.

Results

All 100 isolates were cultured after anaerobic incubation for 48h.
Isolates examined were:

Clostridium perfringens (2 strains)
C. paraputrificum
C. butyricum / beijerinckii
C. septicum
C. tetani
C. difficile
C. clostridioforme
C. bifermentans
C. novyi type A
C. ramosum
C. cadaveris
C. sordellii
C. sporogenes
C. glycolicum
Bacteroides fragilis (4)
B. thetaiotaomicron (3)
Peptostreptococcus magnus (2)
Peptostrep. Asaccharolyticus (2)
Peptostrep. Productus (2)
Peptostrep. Micros (2)
Peptostrep. Anaerobius (2)
Peptostrep. Species (3)
Streptococcus mutans
Staphylococcus saccharolyticus
Eggerthella lenta (Eubacterium lentum, 3)
Eubacterium aerofaciens (2)
Actinomyces israelii (2)
A. naeslundii (2)
A. odontolyticus
A. turicensis
A. viscosis
Actinomyces species (2)

B. distasonis (2)
B. uniformis
B. opvatus (2)
B. vulgaris
B. splanchnicus (2)
Prevotella loescheii
Prev. denticola
Prev. melaninogenica (2)
Prev. oris
Prevotella species (2)
Porphyromomas asaccharolytica
Porph. endodontalis
Porph. levii
Porphyromonas species
Fusobacterium necrophorum (3)
F. nucleatum (4)
F. varium
F. russii
F. naviforme
A. gerencseriae
Bifidobacterium longum
Bif. Animalis group
Bifidobacterium species (2)
Propionibacterium acnes (2)
Prop. Propionicum
Prop. Granulosum
Lactobacillus acidophilus
Campylobacter recta
Camp. gracilis
Camp. Ureolyticus (2)
Campylobacter species
Bilophila wadsworthia
Veillonella parvula (2)

Dr. Val Hall,
Anaerobe Reference Unit, PHLS, University Hospital of Wales, Cardiff, CF14 4X

Results and Discussion

The sampling procedure chosen for the trial was deliberately stringent as it included a dilution step which would not normally be part of the recovery of a strain from cryogenic storage. Survival and recovery of fastidious anaerobes with this protocol, therefore, is a more rigorous test of the system, and makes the results more meaningful.

The overall performance of the Microbank™ preservation system for anaerobes was highly satisfactory. Although variations in recovery are apparent between samples, these are probably due to a combination of heterogeneity of inoculum and sampling error. There was no evidence of a gradual decline in recovery over time as compared to the control.

Three organisms failed to survive the trial period; these were *Actinomyces odontolyticus, Actinomyces israelii* and *Prevotella Intermedia*. The latter two also failed in the control vial, however, and the former was contaminated with a *P.acnes,* presumably at the date of freezing.

In light of these results the Anaerobe Reference Unit has adopted the Microbank™ system for the preservation of strains in its culture collection.

Reference
Microbank™ Storage (5 years) Trial for Anaerobes

Method and Materials

One bead from each vial will be inoculated onto Fastidious Anaerobe agar (Lab M, Bury) with 5% horse blood, and spread for single colonies. Plates will be incubated promptly in a an anaerobic chamber (Concept 300 or Concept Plus, Fred Baker Scientific, UK) at 37°C for 48 hours. On removal from the chamber, cultures will be examined for growth consistent with the intended isolate. Cultures yielding no growth will be incubated for a further 3 days and re-examined.

Results

Growth will be recorded as + (intended isolate recovered) or - (isolate not recovered).
Original inocula were not standardised, therefore, quantitation of growth would be fairly meaningless. Besides, for our purposes, density of growth is unimportant as long as the isolate is recoverable.

MICROBANK™ STORAGE (5 YEAR) TRIAL – RESULTS

PHLS Anaerobe Reference Unit, University Hospital of Wales, Cardiff CF4 4XW

ARU ref.	Organism	Growth	Comments
R5555	*Clostridium perfringens*	+	
R5558	*C.paraputrificum*	+	
R5559	*C.butricum/beijerinckii*	+	
R5560	*C.septicum*	+	
R5572	*C.tetani*	+	
R5573	*C.difficile*	+	
R5584	*C.clostridioforme*	+	
R5586	*C.perfringens*	+	
R5601	*C.bifermentans*	+	
R5606	*C.novyi type A*	+	
R5628	*C.ramosum*	+	
R5635	*C.cadaveris*	+	
R5642	*C.sordellii*	+	
R5759	*C.sporogenes*	+	
R5760	*C. glycolicum*	+	
R5570	*Bacteroides thetaiotaomicron*	+	
R5587	*B.fragilis*	+	
R5589	*B.fragilis (metronidazole resist.)*	+	
R5600	*B.distasonis*	+	
R5620	*B.fragilis*	+	

MICROBANK™ STORAGE (5 YEAR) TRIAL – RESULTS

PHLS Anaerobe Reference Unit, University Hospital of Wales, Cardiff CF4 4XW

ARU ref.	Organism	Growth	Comments
R5631	*B.uniformis*	+	
R5745	*B.ovatus*	+	
R5755	*B.thetaiotaomicron*	+	
R5762	*B.distasonis*	+	
R5791	*B. vulgaris*	+	
R5801	*B. ovatus*	+	
R5867	*B. splanchnicus*	+	
R5868	*B. splanchnicus*	+	
R5933	*B. thetaiotaomicron*	+	
R5956	*B. fragilis*	+	
R5783	*Prevotella loescheii*	+	
R5836	*Prevotella species*	+	
R5839	*Prevotella species*	+	
R5954	*Prev. denticola*	+	
R5955	*Prev. melaninogenica*	+	light growth after 3 days
R5974	*Prev. melaninogenica*	+	light growth after 3 days
R6080	*Prev. oris*	+	
R5550	*Porphyromonas asaccharolytica*	+	
R5807	*Porph. Endodontalis*	+	
R5838	*Porphyromonas species*	+	

MICROBANK™ STORAGE (5 YEAR) TRIAL – RESULTS

PHLS Anaerobe Reference Unit, University Hospital of Wales, Cardiff CF4 4XW

ARU ref.	Organism	Growth	Comments
R5995	*Porph. Endodontalis*	+	
R6079	*Porph. Levii*	+	
R5565	*Fusobacterium necrophorum*	+	
R5585	*F. necrophorum*	+	
R5641	*F. varium*	+	
R5716	*F. russii*	+	
R5748	*F. nucleatum*	+	
R5769	*F. naviforme*	+	
R5778	*F. necrophorum*	+	
R6000	*F. nucleatum*	+	
R6003	*F. nucleatum*	+	
R6066	*F. nucleatum*	+	Light growth after 3 days
R5562	*Peptostrep. Asaccharolyticus*	+	
R5563	*Peptostreptococcus species*	+	
R5622	*Peptostreptococcus species*	+	
R5624	*Peptostreptococcus species*	+	
R5630	*Peptostreptococcus productus*	+	
R5668	*Staphylococcus saccharolyticus*	+	
R5720	*Peptostreptococcus magnus*	+	
R5761	*Peptostreptococcus productus*	+	

MICROBANK™ STORAGE (5 YEAR) TRIAL – RESULTS

PHLS Anaerobe Reference Unit, University Hospital of Wales, Cardiff CF4 4XW

ARU ref.	Organism	Growth	Comments
R5767	*Peptostrept. Magnus*	+	
R5805	*Peptostrept. micros*	+	
R5806	*Peptostrept.anaerobius*	+	
R5826	*Streptococcus mutans*	+	
R5840	*Peptostrept. anaerobius*	+	
R5927	*Peptostrep .asaccharolyticus*	+	
R5997	*Peptostrept. micros*	+	
R5569	*Eubacterium aerofaciens*	+	
R5598	*E. lentum*	+	
R5670	*E. lentum*	+	
R5800	*E. aerofaciens*	+	
R5837	*Eu. Lentum*	+	
R5552	*Actinomyces naeslundii*	+	
R5554	*A. israelii*	+	
R5557	*A. israelii*	+	
R5568	*A. odontolyticus*	+	
R5571	*Actinomyces species*	+	
R5619	*Actinomyces species*	+	
R5634	*A. naeslundii*	+	
R5639	*A. turicensis*	+	

MICROBANK™ STORAGE (5 YEAR) TRIAL – RESULTS

PHLS Anaerobe Reference Unit, University Hospital of Wales, Cardiff CF4 4XW

ARU ref.	Organism	Growth	Comments
R5718	*Actinomyces viscosis*	+	
R5774	*A. gerencseriae*	+	
R5556	*Bifodobacterium species*	+	
R5588	*Bifodobacterium species*	+	
R5824	*Bif. Longum*	+	
R5921	*Bif. Animalis group*	+	
R5561	*Propionibacterium acnes*	+	
R5567	*Prop. Propionicum*	+	
R5671	*Prop. granulosum*	+	
R5764	*Prop. Acnes*	+	
R6085	*Lactobacillus acidophilus*	+	
R6097	*L. acidophilus*	+	
R5551	*Campylobacter recta*	+	
R5669	*Camp. gracillis*	+	
R5738	*Campylobacter species*	+	
R5756	*Camp. ureolylicus*	+	
R6001	*Camp .ureolyticus*	+	
R6043	*Bilophilia wadsworthia*	+	
R5675	*Veillonella parvula*	+	
R5848	*V.parvula*	+	

MICROBANK™ STORAGE (5 YEAR) TRIAL

PHLS Anaerobe Reference Unit, University Hospital of Wales, Cardiff CF4 4XW

Bacterial strains (n = 100) frozen in 1993:

ARU ref	Organism	ARU ref	Organism
R5555	*Clostridium perfringens*	R5558	*C. paraputrificum*
R5559	*C. butyricum/beijerinckii*	R5560	*C. septicum*
R5572	*C. tetani*	R5573	*C. difficile*
R5584	*C. clostridioforme*	R5586	*C. perfringens*
R5601	*C. bifermentans*	R5606	*C. novyi type A*
R5628	*C. ramosum*	R5635	*C. cada veris*
R5642	*C. sordellii*	R5759	*C. sporogenes*
R5760	*C. glycolicum*	R5770	*B. thetaiotaomicron*
R5587	*B. fragilis*	R5589	*B. fragilis (metronidazole)*
R5600	*B. distasonis*	R5620	*B. fragilis*
R5631	*B. uniformis*	R5745	*B. ovatus*
R5755	*B. thetaiotaomicron*	R5762	*B. distasonis*
R5791	*B. vulgaris*	R5801	*B. ovatus*
R5867	*B. splanchnicus*	R5868	*B. splanchnicus*
R5933	*B. thetaiotaomicron*	R5956	*B. fragilis*
R5783	*Prevotella loescheii*	R5836	*Prevotella species*
R5839	*Prevotella species*	R5954	*Prev. denticola*
R5955	*Prev. melaninogenica*	R5974	*Prev. melaninogenica*
R6080	*Prev. oris*	R5550	*Porphyromonas asaccharolytica*
R5807	*Porph. endodontalis*	R5838	*Porph. species*
R5995	*Porph. endodontalis*	R6079	*Porph. levii*
R5565	*Fusobacterium necrophorum*	R5585	*F. necrophorum*
R5641	*F. varium*	R5716	*F. russii*
R5748	*F. nucleatum*	R5769	*F. naviforme*
R5778	*F. necrophorum*	R6000	*F. nucleatum*
R6003	*F. nucleatum*	R6066	*F. nucleatum*
R5562	*Peptostreptococcus asaccharolyticus*	R5563	*Peptostreptococcus species*
R5622	*Peptostreptococcus species*	R5624	*Peptostreptococcus species*
R5630	*Ps. productus*	R5668	*Staphylococcus saccharolyticus*
R5720	*Ps. magnus*	R5761	*Ps. Productus*
R5767	*Ps. magnus*	R5805	*Ps. Micros*
R5806	*Ps. anaerobius*	R5826	*Streptococcus mutans*
R5840	*Ps. anaerobius*	R5927	*Ps. Asaccharolyticus*
R5997	*Ps. micros*		

ARU ref	Organism	ARU ref	Organism
R5569	*Eubacterium aerofaciens*	R5598	*E. lentum*
R5670	*E. lentum*	R5800	*E. aerofaciens*
R5837	*E. lentum*	R5552	*Actinomyces naeslundii*
R5554	*A. israelii*	R5557	*A. israelii*
R5568	*A. odontolyticus*	R5571	*Actinomyces species*
R5619	*Actinomyces species*	R5634	*A. naeslundii*
R5639	*A. turicensis*	R5718	*A. viscosus*
R5774	*A. gerencseriae*	R5556	*Bifidobacterium species*
R5588	*Bifidobacterium species*	R5824	*Bif. longum*
R5921	*Bif. animalis group*	R5561	*Propionibacterium acnes*
R5567	*Prop. propionicum*	R5671	*P. granulosum*
R5764	*Prop. acnes*	R6085	*Lactobacillus acidophilus*
R6097	*L. acidophilus*	R5551	*Campylobacter recta*
R5669	*Camp. gracilis*	R5738	*Campylobacter species*
R5756	*Camp. ureolyticus*	R6001	*Camp. ureolyticus*
R6043	*Bilophila wadsworthia*	R5675	*Veillonella parvula*
R5848	*V. parvula*		

Method and Materials

One bead from each vial will be inoculated onto Fastidious Anaerobe Agar (Lab M, Bury) with 5% horse blood, and spread for single colonies. Plates will be incubated promptly in an anaerobic chamber (Concept 300 or Concept Plus, Fred Baker Scientific, UK) at 37°C for 48 hours. On removal from the chamber, cultures will be examined for growth consistent with the intended isolate. Cultures yielding no growth will be incubated for a further 3 days and re-examined.

Results

Growth will be recorded as + (intended isolate recovered) or - (isolate not recovered). Original inocula were not standardized, therefore, quantitation of growth would be fairly meaningless. Besides, for our purposes, density of growth is unimportant as long as the Isolate is recoverable.

Reference

Reprints from **ABSTRACTS FOR THE ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, 1994 23 – 27 May 1994 Las Vegas, Nevada**.

C-352 Storage and Repeated Recovery of *Neisseria gonorrhoeae* using Pro-Lab Microbank™

H. YOUNG* and A. MOYES
University of Edinburgh, Edinburgh, Scotland

Existing methods of storing *Neisseria gonorrhoeae* such as lyophilisation or freezing in liquid nitrogen are not ideal. The aim of this study was to evaluate the Microbank™ system for storage and multiple retrieval of *N. gonorrhoeae*. In this system organisms are harvested in cryopreservative, added to a vial containing 20 small porous beads, excess liquid removed, and the vial frozen at -70°C.

100 gonococcal isolates, representing 8 protein IA serovars and 14 protein IB serovars, were included. Each isolate was cultured overnight on modified New York City medium and the growth harvested into a cryovial. Cryovials were immediately placed at -70°C. A bead from each cryovial was removed and cultured on modified New York City medium at monthly intervals. Sampling the 100 cultures over 18 monthly retrievals gave an overall recovery rate of 98.8% (1778/1800): the 22 failures represented 10 separate isolates. Failure was not related to length of storage as all 100 isolates were recovered at month 18.

We concluded that Pro-Lab Microbank™ is a highly effective and convenient system for storage of *Neisseria gonorrhoeae*, particularly when multiple retrieval is required. The system offers many advantages over conventional lyophilisation.

Reference

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Storage and Repeated Recovery of *Neisseria gonorrhoeae* using Microbank™ Cryovials

A. MOYES and H. YOUNG

Scottish Gonococcal Reference Laboratory, Department of Medical Microbiology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG. Scotland, UK

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Abstract: One hundred gonoccocal isolates, representing eight protein IA serovars and 14 protein IB serovars, were stored at -70°C using the Pro-Lab Microbank™ cryovial storage system. At monthly intervals a bead from each cryovial was removed and cultured on modified New York City medium. The overall recovery rate was 98.6% (2365/2400), the 35 failures representing 13 separate isolates. There was a small but significant decrease in recovery in the last 12 months of the trial (97.8%) compared with the first 12 months (99.8%), which may have been due to a sampling problem rather than a temporal phenomenon Failures were significantly associated with minor serovars, suggesting that the transmissibility/viability of minor serovars may be lower than that of common serovars and could be a significant factor in the epidemiology of gonococcal infection.

We conclude that Pro-Lab Microbank™ cryovials provide a highly effective and convenient system for storage of *Neisseria gonorrhoeae, particularly when multiple retrieval is required, and the system offers many advantages over conventional lyophilisation.*

Key words: Cryopreservation. *Neisseria gonorrhoeae.* Serotyping.

Introduction

Due to the fastidious nature of *Neisseria gonorrhoeae* a simple, inexpensive and efficient system for the storage and recovery of clinical isolates and quality control strains is required for good clinical laboratory practice, in research and for epidemiological studies. Various methods such as use of cooked meat broth, lyophilisation or freezing in liquid nitrogen[1] are available for the storage of bacteria but none is ideal, particularly for gonococci. The limitations associated with these methods are the variable recovery of bacteria, the time and inconvenience involved in the preparation and maintenance of cultures and the financial cost in the purchase and maintenance of expensive equipment.

The technique of storing organisms at -70°C described by Nagel and Lawrence in 1971[2] has given rise to simple and convenient commercial storage systems such as the Pro-Lab Microbank™[3] which uses coloured beads in a 'cryovial' containing cryopreservative fluid. After inoculation and storage a single bead can be removed to inoculate culture media.

We examined the Microbank™ system with a view to its overall convenience of use for storage and recovery. The recovery rate after medium-term storage and repeated sampling was analysed and an evaluation made of the efficacy of recovery in relation to the spectrum of antigenic types (serovars) of gonococci that occur in nature.

Materials and Methods

Bacterial strains. One hundred clinical isolates of *N. gonorrhoeae*, including 30 penicillinase-producing *N. gonorrhoeae* were included in the study. These strains represented the wide variety of antigenic types of gonococci encountered in natural infection and comprised 26 serogroup IA strains covering eight different serovars, 61 serogroup IB strains covering 14 different serovars and 13 serogroup IB strains which were non-typeable with the standard monoclonal antibody serotyping panel.[4]

Preservation and storage of strains. Using a sterile cotton bud, gonococcal colonies were harvested from an 18-24 hour culture on modified New York City medium[5] and a suspension made in the cryopreservative fluid of the cryovial approximately equivalent to a McFarland No. 4 standard. The inoculated vial was closed and the contents inverted 4-5 times to coat the beads with bacteria. Excess cryopreservative fluid was removed with a sterile pastette. The vial closed and immediately placed in a -70°C freezer.

Retrieval of bacteria. All 100 gonococcal isolates were sampled each month for 24 consecutive months. Twenty cryovials at a time were removed from the -70°C freezer, using an aluminium transfer block[3] to retain a low temperature. Using sterile forceps a single bead was removed from the cryovial, placed onto the surface of a culture place containing modified New York City medium, and allowed to thaw. A sterile loop was used to streak out the area around the bead to obtain separate colonies and the culture plates incubated for 48 hours in a carbon dioxide-enriched atmosphere.

Serotyping. Sereotyping of the gonococcal strains was performed using the Genetic Systems[6] panel of monoclonal antibodies.[7]

Statistical analysis. The chi-squared test was used for all statistical analysis.

Results

As shown in **Table 1**, the overall recovery rate from 2400 retrievals for the 24 months of the trial was 98.6% (2365/2400), and all strains were recovered in eight of the 24 months (100% recovery). The recovery rate for the remaining months ranged from 99% to 96%: 99% in five of the months, 98% in five of the months, 97% in four of the months and 96% in two of the months. Although the recovery rate was extremely good there were 0.8% (9/1191) failures in the first 12 months compared with 2.2% (26/1174) in the last 12 months – a significant difference ($x^2 = 8.4$; $P<0.01$).

Thirteen separate isolates accounted for the 35 (1.5%) failures and the distribution of these failures by serovar and month is shown in **Table 2.** Five isolates failed on only one occasion, four on two occasions, two isolates on five occasions and two isolates on six occasions.

The distribution of the 13 serovar failures in relation to the total number of isolates for each of the 23 sereovars tested is given in **Table 3.** Although there were only three serovar IB15 isolates, each one failed on at least one occasion and together they accounted for 37.1% (13/35) of the total failures. Sereovars IA16 and IB25 were also associated with multiple failures on five or more occasions **(Table 2).**

Thirty-five isolates comprising serovars IA02, IA06, IB01, IB02 and IB03, classified as major serovars from continuous prevalence studies in our geographical area,[8] accounted for only 2.0% (1/35) of the failures. The remaining 65 isolates, classified as minor serovars, accounted for 18.5% (12/65) of the failures – a significant difference ($x^2 = 4.9$; $P<0.05$).

Table 1 – Monthly recovery rate for 100 gonococcal isolates

Month	No. of Recovered Strains	No. of Failures
1	100	0
2	100	0
3	100	0
4	99	1
5	100	0
6	100	0
7	100	0
8	100	0
9	99	1
10	98	2
11	98	2
12	97	3
13	99	1
14	97	3
15	97	3
16	98	2
17	96	4
18	100	0
19	97	3
20	98	2
21	99	1
22	99	1
23	96	4
24	98	2
Total	**2365 (98.6%)**	**35 (1.5%)**

Table 2 – Distribution of 35 failures by serovar and month

Serovar	Month	Serovar	Month
IA05	15,16	IB15	13,14,15,20,23,24
IA16	11,15,17,19,23	IB19	23
IA21	17	IB25	4,9,10,12,14,17
IA25	17,19	IB29	19
IB02	21	IB00	10,23
IB15	12,24	IB00	12
IB15	11,14,16,20,22		

IB00 (Non-typeable strain)

Table 3 – Distribution of 13 failures in relation to individual serovars

Serovar	No. of Isolates	No. of Failures
IA02	6	0
IA04	3	0
IA05	2	1
IA06	5	0
IA07	1	0
IA16	4	1
IA21	3	1
IA25	2	1
IB01	10	0
IB02	7	1
IB03	7	0
IB05	2	0
IB06	8	0
IB07	5	0
IB08	4	0
IB15	3	3
IB17	7	0
IB19	2	1
IB25	2	1
IB26	1	0
IB29	2	1
IB31	1	0
IB00	13	2

IB00 (Non-typeable strain)

Discussion

Lyophilisation has long been accepted as the 'gold standard' method for the preservation of microorganisms, but the high cost in equipment and processing time precludes its use in many routine laboratories. Modern technology has made -70°C facilities readily available and the small capacity required to store large numbers of isolates makes cryovial storage systems extremely convenient for the clinical laboratory which requires easy access to strains. Concerns over refrigeration failure and the subsequent loss of valuable strains can be alleviated by fitting carbon dioxide back-up systems, designed to activate at a pre-set temperature to the freezer.[9]

Nagel and Lawrence[2] first described a method for the preservation of multiple replicate units of bacteria using sterile glass beads and a mixture of equal parts of broth culture and horse blood allowing storage of at least 200 beads in a plastic tube at -70°C. In a subsequent study Feltham *et al*[10] used different concentrations of cryoprotectants in the storage media used to make the bacterial suspensions, and stored the beads at -70°C. They observed a reduction in the number of viable bacteria with nutrient broth containing 15% dimethyl sulphoxide. Nutrient broth supplemented with either: 10% dimethyl sulphoxide, 10% glycerol or 15% glycerol, showed no such reductions. In a further study White and Sand[11] demonstrated the viability of organisms after storage at -76°C for two years, using glass beads and brain-heart infusion broth containing 10% glycerol as the emulsifying fluid.

In this study we have shown that the Microbank™ system offers a simple commercially available system for medium-term storage and multiple recovery of *N. gonorrhoeae.* The overall recovery rate of 98.6% is extremely good and, together with the ability to sample up to 25 times, represents substantial cost benefits. The failure of four isolates to grow at least five times each may be associated with the strains, or may be a simple physical problem of insufficient primary inoculum in these vials. The overall recovery rate could possibly be improved with the use of special recovery medium. Morton and Smith[12] advocated the use of a solution of 20% sucrose in phosphate-buffered saline for the recovery of fastidious organisms such as *Neisseria* spp, though clearly this is not essential for the vast majority of gonococcal isolates.

There was a small but significant decrease in recovery in the last 12 months of the trial, which may reflect a sampling problem rather than a temporal phenomenon and further long-term studies are underway to differentiate between these possibilities. The finding that failures were significantly associated with minor sereovars suggests that the transmission/viability of minor serovars may be lower than that of common serovars and could be a significant factor in the overall epidemiology of gonococcal infection. The selective loss of minor serovars on storage could also lead to a bias in epidemiological studies based on isolates that have been stored for some time.

References

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LONG TERM STORAGE OF FASTIDIOUS CAMPYLOBACTER AND HELIOBACTER USING MICROBANK™

K. Illingworth, E Le Roux and A J Lastovica, Dept. of Medical Microbiology, Red Cross Childens Hospital, Rondesbosch 7700, Cape Town, South Africa

Long term storage of fastidious Campylobacter and Helicobacter has proved to be difficult, as various workers have described low recovery rates on revitralizing freeze-dried cultures and other methods of preservation. We have used the Microbank™ (Pro-Lab Diagnostics, Texas, USA) system, in which porous beads act as carriers to support micro-organisms.

Campylobacter mucosalis, C.concisus, C.hyointestinalis, C.curvus, H.pylori and Hfennelliae were tested in this system. Two or three day culture grown on tryptose blood agar plates (Oxoid CM233) under 11' enriched microaerophilic conditions were inoculated into the Microbank™ media and then stored at -70°C.

For revitalization, one bead was placed on the surface of a blood agar plate, allowed to thaw and gently rolled over the surface. Plates were incubated in an II' enriched microaerophilic environment at 37°C for up to 7 days.

Twenty-two clinical and reference cultures of C.mucosalis and C.conscisus were successfully revitalized from Microbank™ storage at 3 weeks, 4 months and 6 months. Five isolates of C.hyointestinalis were successfully revitalized after 7 months. The type strain of C.curvus, NCTC 11649 was revitalized at 3 weeks, 3 months and 6 months.

Thirty nine of 41 (95%) clinical and reference isolates of H. pylori were revitalized at 2 weeks, 2 months and 8 months. Concurrent revitalization of freeze-dried cultures of these H.pylori isolates indicated that only 10 of 31 (32%) strains were viable.

Seventeen of 24 (71%) H.fennelliae clinical and reference cultures were viable. Fourteen isolates were revitalized after 6 months, and 3 isolates after 17 to 21 months. The initial inoculums must be heavy, and revitalization should be in a hydrogen enriched microareophilic atmosphere. The loss of viability in some of the H.pylori and H.fennelliae isolates is attributed to too sparse an inoculum.

The Microbank™ system provides a very simple solution to long term storage of fastidious Campylobacter and Helicobacter strains. As this commercial preservation system is freely available, it obliviates the need for specialized media and procedures. Additional testing is required for longer term storage in the Microbank™ system of these medically important micro-organisms.

Reference

Long term Storage of Salmonella isolates submitted to the Salmonella Reference Typing Laboratory, Veterinary Laboratory Agency, Weybridge, Surrey using the Microbank™ Bacterial Storage and Retrieval System.

Microbank™ Bacterial Storage Record 17/05/1999

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
1	Agona	1	4,12	S7134/92FH	05/04/1995	05/04/1995			
2	amager	1	3,10	S4338/88FH	05/04/1995	05/04/1995			
3	anatum	1	3,10	S4655/91FH	20/05/1992	20/05/1992			
4	Artis	2	56	VERIFIED	23/04/1992	23/04/1992			
5	bilthoven	2	47	LASSWADE	23/04/1992	23/04/1992			
6	bredeney + ST394	1	1,4,12	S4743/91EO	20/05/1992	20/05/1992			
7	16:mt:z42	2	16	9008/92AZ	05/04/1995	05/04/1995			
8	Derby	1	1,4,12	S6623/92FI	29/05/1992	29/05/1992			
9	Dublin	1	9,12	S7693/91CC	20/05/1992	20/05/1992			
10	enteritidis + st506	1	9,12	S4546/91BC	29/05/1992	29/05/1992			
11	windermere	1	39	VERIFIED	16/10/1998	16/10/1998			
12	vinohrady	1	28	VERIFIED	20/03/1998	20/02/1998			
13	Hato	1	4,12	VERIFIED	31/08/1995	31/08/1995			
14	Lille	1	6,7	S4598/91FH	20/05/1992	20/05/1992			
15	livingstone	1	6,7	S4392/91BC	20/05/1992	20/05/1992			
16	gallinarum	1	9,12	VERIFIED	16/04/1998	16/04/1998			
17	typhi murium + ST38	1	4,5,12	S9795/91BD	20/05/1992	20/05/1992			
18	panama	1	9,12	S6570/91AS	20/05/1992	20/05/1992			
19	kiambu	1	4,12	LASSWADE	05/04/1995	05/04/1995			
20	thompson	1	6,7	S6554/93CC	20/05/1992	20/05/1992			
21	durham	1	13,23	S3922/90CC	05/04/1995	05/04/1995			
22	paratyphi B (Cat.3)	1	4,5,12	S3922/90CC					
23	60:a:-	2	60	VERIFIED	22/04/1992	22/04/1992			
24	indiana	1	1,4,12	S4123/91BT	20/05/1992	20/05/1992			
25	meleagridis	1	3,10	S5592/91SS	20/05/1992	20/05/1992			
26	heidelberg	1	4,5,12	S5813/91CC	20/05/1992	20/05/1992			
27	ochiogo	1	3,19	VERIFIED	06/01/1998	06/01/1998			
28	montevideo	1	6,7	S4806/91FH	31/08/1995	31/08/1995			
29	senftenberg	1	1,3,19	S8997/93BC	13/09/1993	13/09/1993			
30	infantis + ST425	1	6,7	S4533/91BC	05/04/1995	05/04/1995			
31	cubana	1	13,23	S4580/91FH	05/04/1995	05/04/1995			
32	Isangi	1	6,7	VERIFIED	06/01/1998	06/01/1998			
33	hithergreen	1	16	VERFIED	20/03/1998	20/03/1998			
34	bareilly	1	6,7	S13604/90EC	30/05/1996	30/05/1996			
35	nagoya	1	6,8	S3997/94BD	03/06/1994	03/06/1994			
36	saint paul	1	1,4,5,12	S3836/90CC	05/04/1995	05/04/1995			
37	monschaui	1	35	S8208/92FH	11/09/1996	11/09/1996			
38	Deleted 8/1/98				07/07/1992	07/07/1992			
39	kentucky + ST83	1	8,20	S9708/92BT	24/08/1992	24/08/1992			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
40	abortus ovis	1	4,12	LASSWADE	19/01/1993	19/01/1993			
41	tournai	1	(1),3,15	VERIFIED	20/03/1998	20/03/1998			
42	4,12:d:-	1	4,12	S4277/91EO	05/04/1995	05/04/1995			
43	nyborg	1	3,10	VERIFIED	31/08/1995	31/08/1995			
44	muenchen	1	6,8	S11345/90AZ	05/04/1995	05/04/1995			
45	san diego	1	4,5,12	S886/89DP	10/08/1992	10/8/1992			
46	chester	1	4,12	S3300/91AD	05/04/1995	05/04/1995			
47	gallinarum	1	9,12	VERIFIED	20/03/1998	20/03/1998			
48	langford	1	28	VERIFIED	31/08/1995	31/08/1995			
49	Deleted 15/10/98								
50	richmond	1	6,7	VERIFIED	16/10/1998	16/10/1998			
51	virginia	1	8	VERIFIED	31/08/1995	31/08/1995			
52	weltevreden	1	3,10	VERIFIED	04/05/1995	04/05/1995			
53	Lamin	1	3,10	LASSWADE	22/10/1992	22/10/1992			
54	gaminara	1	16	VERIFIED	31/08/1995	31/08/1995			
55	Oxford	1	3,10	VERIFIED	31/08/1995	31/08/1995			
56	wangata	1	9,12	S6956/92BC	04/05/1995	04/05/1995			
57	tennessee	1	6,7	S4783/91BC	04/05/1995	04/05/1995			
58	reading	1	4,12	S812/90BT	04/05/1995	04/05/1995			
59	agama	1	4,12	S5723/91CC	04/05/1995	04/05/1995			
60	stanley	1	4,5,12	VERIFIED	03/08/1995	03/08/1995			
61	arizona	3	65	S11613/96AS	28/11/1996	28/11/1996			
62	braenderup	1	6,7	S7639/92EG	16/07/1992	16/07/1992			
63	hadar	1	6,8	S6106/91BT	04/05/1995	04/05/1995			
64	virchow	1	6,7	S5967/91BC	04/05/1995	04/05/1995			
65	newport	1	6,8	S4430/91/BT	04/05/1995	04/05/1995			
66	hvittingfoss	1	16	S9353/91FH	09/07/1992	09/07/1992			
67	amsterdam	1	3,10	S596/91FH	04/05/1995	04/05/1995			
68	oranienburg	1	6,7	S67789OFI	10/08/1992	10/08/1992			
69	Haardt	1	8	LASSWADE	08/01/1993	08/01/1993			
70	drypool	1	3,15	S5730/91AS	04/05/1995	04/05/1995			
71	tyresoe	1	4,12	VERIFIED	20/11/1996	20/11/1996			
72	arizona	3b	50	LASSWADE	21/11/1995	21/11/1995			
73	dessau	1	(1),3,15,(19)	VERIFIED	19/07/1995	19/07/1995			
74	albany	1	8,20	S7142/91FH	04/05/1995	04/05/1995			
75	california	1	4,12	S3485/91FH	04/05/1995	04/05/1995			
76	Emek	1	8,20	S4942/92FH	04/05/1995	04/05/1995			
77	shipley	1	8,20	S2122/91DF	04/05/1995	04/05/1995			
78	newhaw	1	3,15	VERIFIED	06/01/1998	06/01/1998			
79	kinshasa	1	3,15	S3536/89BT	11/05/1995	11/05/1995			
80	bovis morbificans	1	6,8	LASSWADE	10/08/1992	10/08/1992			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
81	cerro	1	6,18	S5582/9/FH	11/05/1995	11/05/1995			
82	Dugbe	1	45	S10190/92FH	26/01/1996	26/01/1996			
83	kentucky +ST39	1	8,20	S2610/95/OT	19/01/1996	19/01/1996			
84	eastbourne	1	9,12	VERIFIED	20/03/1998	20/03/1998			
85	arizona	3b	35	S3701/9/FH	08/04/1992	08/04/1992			
86	coley park	1	6,7	S7795/92FH	11/05/1995	11/05/1995			
87	jukestown	1	13,23	VERIFIED	20/03/1998	20/02/1998			
88	Give	1	3,10	S6203/91fh	11/05/1995	11/05/1995			
89	chicago	1	28	S6744/96FI	10/05/1995	10/09/1995			
90	zanzibar	1	3,10	VERIFIED	20/11/1996	20/11/1996			
91	newington	1	3,15	S6108/91BT	11/05/1995	11/05/1995			
92	Deleted 19/03/98								
93	urbana	1	30	S7808/92FH	09/07/1992	09/07/1992			
94	Poona	1	13,22	S6666/91FH	10/01/1996	10/01/1996			
95	adelaide	1	35	S4348/88FH	09/01/1992	09/07/1992			
96	liverpool	1	1,3,19	VERIFIED	20/03/1998	20/03/1998			
97	godesberg	1	30	S6972/91EO	11/05/1995	11/05/1995			
98	Perth	1	38	VERIFIED	08/01/1998	08/01/1998			
99	bere	1	47	VERIFIED	07/12/1993	07/12/1993			
100	mishmar haemek	1	13,23	S1195/92FI	11/05/1995	11/05/1995			
101	potsdam	1	6,7	S4584/92FH	11/05/1995	11/05/1995			
102	hull	1	16	VERIFIED	20/03/1998	20/03/1998			
103	takoradi	1	6,8	S1184/92AS	11/05/1995	11/05/1995			
104	London + ST297	1	3,10 S.rough	S7368/91FH	11/05/1995	11/05/1995			
105	taksony	1	1,3,19	S4431/91BT	11/05/1995	11/05/1995			
106	tado	1	8,20	VERIFIED	30/11/1995	30/11/1995			
107	binza	1	3,15	S4559/91EO	11/05/1995	11/05/1995			
108	humber	2	53	LASSWADE	11/08/1992	11/08/1992			
109	concord	1	6,7	VERIFIED	08/01/1998	08/01/1998			
110	treforest	1	1,51	LASSWADE	11/05/1995	11/05/1995			
111	corvallis	1	8,20	VERIFIED	20/03/1998	20/03/1998			
112	aba	1	(6),8	VERIFIED	20/03/1998	20/03/1998			
113	tranoroa	1	55	LASSWADE	08/03/1993	08/03/1993			
114	locarno	1	57	LASSWADE	05/10/1992	05/10/1992			
115	orion	1	3,10	S4768/91EO	11/05/1995	11/05/1995			
116	blockley	1	6,8	VERIFIED	20/03/1998	20/03/1998			
117	alachua	1	35	S6293/91FH	11/05/1995	11/05/1995			
118	Basel	2	58	NCTC10310	08/10/1992	08/10/1992			
119	59:(k):- (betioky)	2	59	VERIFIED	16/10/1998	16/10/1998			
120	moscow/blegdam	1	9,12	LASSWADE	05/10/1992	05/10/1992			
121	tallahassee	1	6,8	NCTC7893	07/08/1992	07/08/1992			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
122	brandenburg	1	1,4,12	S1370/90AD	11/05/1995	11/05/1995			
123	guinhon	1	47	LASSWADE	10/08/1992	10/08/1992			
124	michigan	1	17	S128350-COL	11/05/1995	11/05/1995			
125	Florida	1	1,6,14,25	S1381/95AS	10/01/1996	10/01/1996			
126	lexington	1	3,10	S6336/91EO	11/05/1995	11/05/1995			
127	uganda	1	3,10	S4038/90FI	01/07/1992	01/07/1992			
128	grumpensis	1	13,23	NCTC6533161	01/07/1992	01/07/1992			
129	utrecht	1	52	VERIFIED	04/09/1996	04/09/1996			
130	rostock	1	1,9,12	LASSWADE	28/07/1992	28/07/1992			
131	dusseldorf	1	6,8	NCTC6262	07/08/1992	07/08/1992			
132	champaign	1	39	LASSWADE	16/07/1992	16/07/1992			
133	Yolo	1	35	VERIFIED	20/03/1998	20/03/1998			
134	canoga	1	(3),(15),34	VERIFIED	19/07/1995	19/07/1995			
135	waycross	1	41	S4842/90MO	16/07/1992	16/07/1992			
136	rutgers	1	3,10	NCTC9794	07/08/1992	07/08/1992			
137	Guinea	1	44	LASSWADE	11/08/1992	11/08/1992			
138	schwarzengrund	1	1,4,12,27	S4034/91FH	11/05/1995	11/05/1995			
139	28:mt:-	2	28	VERIFIED	20/03/1998	20/03/1998			
140	Inverness	1	38	LASSWADE	16/07/1992	16/07/1992			
141	butantan	1	3,10	S4453/92FI	11/05/1995	11/05/1995			
142	niloese	1	3,19	LASSWADE	23/09/1992	23/09/1992			
143	landwasser	1	3,10	9195/92FH	14/09/1993	14/09/1993			
144	haarlem	2	(9),46	VERIFIED	10/01/1997	10/01/1997			
145	niarembe	1	44	VERIFIED	14/09/1993	14/09/1993			
146	bedford	1	3,19	VERIFIED	16/04/1998	16/04/1998			
147	(greenside)	2	50	VERIFIED					
148	c-suis varkunzendorf	1	6,7	S242/90PP	12/05/1995	12/05/1995			
149	abortus equi	1	4,12	LASSWADE	29/07/1992	29/07/1992			
150	Abony	1	4,12,27	VERIFIED	20/03/1998	20/03/1998			
151	wyldergreen	1	13,23	VERIFIED	20/03/1998	20/03/1998			
152	arizona	3	50	VERIFIED	16/10/1998	16/10/1998			
153	Deleted 19/03/98								
154	chittagong	1	1,3,10,19	NCTC7374	23/09/1992	23/09/1992			
155	wandsbek	2	21	S10449/92AS	14/09/1993	14/09/1993			
156	kimuenza	1	4,12	S9842/91FH	14/09/1993	14/09/1993			
157	malsatt	1	16	S8161/91FH	14/09/1993	14/09/1993			
158	senftenberg	1	3,19	WHO87K	08/04/1994	08/04/1994	30/06/1998		
159	Deleted 19/03/98								
160	chomedey	1	8	VERIFIED	03/03/1998	03/03/1998			
161	duisburg	1	(1),4,12	VERIFIED	20/03/1998	20/03/1998			
162	canastel	2	9,12	S3598/94FI	26/04/1904	26/04/1904			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
163	coein	1	4,5,12	S259/92FH	12/05/1995	12/05/1995			
164	kottbus	1	6,8	VERIFIED	03/03/1998	03/03/1998			
165	hartford	1	6,7	VERIFIED	03/03/1998	03/03/1998			
166	arizona	3a	41	VERIFIED	10/02/1999	10/02/1999			
167	ordonez	1	13,23,	VERIFIED	20/03/1998	20/03/1998			
168	kuessel	1	28	S11245/91/FH	12/05/1995	12/05/1995			
169	jangwani	1	17	VERIFIED	20/03/1998	20/02/1998			
170	falkensee	1	3,10	S1797/90FH	12/05/1991	12/05/1991			
171	stockholm	1	3,10	S6342/91BC	12/05/1991	12/05/1991			
172	goerlitz	1	3,15	S5822/95CC	30/06/1995	30/06/1995			
173	13,23:z:-	1	13,23	VERIFIED	16/04/1998	16/04/1998			
174	haifa	1	(1),4,5,12	VERIFIED	03/03/1998	03/03/1998			
175	manhattan	1	6,8	S3182/92PP	12/05/1995	12/05/1995			
176	Oslo	1	6,7	VERIFIED	16/10/1998	16/10/1998			
177	hindmarsh	1	8	VERIFIED	03/03/1998	03/03/1998			
178	Deleted 19/03/98								
179	Ajiobo	1	13,23	S2259/89FI	09/07/1992	09/07/1992			
180	java	1	4,5,12	S1196/92FI	12/05/1995	12/05/1995			
181	16::-gst:-	2	16	VERIFIED	23/04/1992	23/04/1992			
182	claibornei	1	9,12	S3599/94AS	13/05/1994	13/05/1994			
183	kalamu	1	4,12	VERIFIED	20/03/1998	20/03/1998			
184	litchfield	1	6,8	VERIFIED	03/03/1998	03/03/1998			
185	kapemba	1	9,12	VERIFIED	10/02/1999	10/02/1999			
186	kedougou	1	13,23	S4606/91BC	12/05/1995	12/05/1995			
187	rawash	1	(6),14,18	VERIFIED	06/11/1998	06/11/1998			
188	soesterberg	4	21	VERIFIED	12/05/1992	12/05/1992			
189	offa	1	41	S10229/92AS	12/05/1995	12/05/1995			
190	llandoff	1	1,3,19	S6683/91FH	12/05/1995	12/05/1995	14/10/1998		
191	bradford	1	4,12	VERIFIED	06/11/1998	06/11/1998			
192	mbandaka	1	6,7	S4418/92FH	12/05/1995	12/05/1995			
193	Napoli	1	1,9,12	VERIFIED	10/02/1999	10/02/1999			
194	mons	1	1,4,12	VERIFIED	06/11/1998	06/11/1998			
195	broughton	1	3,19	S423/90FH	12/05/1995	12/05/1995			
196	telhashomer	1	11	S7057/89FH	12/05/1995	12/05/1995			
197	stanleyville	1	1,4,5,12	S15786/91FI	12/05/1995	12/05/1995			
198	ohio	1	6,7	S7339/92/EW	12/05/1995	12/05/1995			
199	pullorum	1	1,9,12	S4165/95BC	26/01/1996	26/01/1996			
200	singapore	1	6,7	VERIFIED	10/02/1999	10/02/1999			
201	rosenthal	1	3,15	VERIFIED	10/02/1999	10/02/1999			
202	9,46:z4z24:-	1	9,46	VERIFIED	17/05/1995	17/05/1995			
203	johannesburg	1	1,40	S9684/91FH	17/05/1995	17/05/1995			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
204	Krefeld	1	3,19	VERIFIED	10/02/1999	10/02/1999			
205	bonariensis	1	6,8	LASSWADE	18/09/1992	18/09/1992			
206	vejle	1	3,10	VERIFIED	10/02/1999	10/02/1999			
207	uccle	1	3,54	NCTC102515	08/01/1992	08/01/1992			
208	luton	2	60	LASSWADE	23/04/1992	23/04/1992			
209	worthington	1	13,23	S6545/91EO	17/05/1995	17/05/1995			
210	Ealing	1	35	S6055/91BC	17/05/1995	17/05/1995			
211	38:z4z23:-	4	38	VERIFIED	31/08/1995	31/08/1995			
212	Telaviv	1	28	LASSWADE	16/07/1992	16/07/1992			
213	Manila	1	3,15	S106/92FI	18/05/1995	18/05/1995			
214	vleuten	1	44	S3705/91FH	18/05/1995	18/05/1995			
215	Gera	1	(1),42	S11408/91FH	18/05/1995	18/05/1995			
216	arizona	3b	61	LASSWADE	08/01/1993	08/01/1993			
217	ashanti	1	28	S3436/91FH	18/05/1995	18/05/1995			
218	Kaolak	1	47	VERIFIED	16/01/1996	16/01/1996			
219	seremban	1	9,12						
220	ruiru	1	21	S6609/91FH	18/05/1995	18/05/1995			
221	molade	1	8,20	S5740/91FH	18/05/1995	18/05/1995			
222	strasbourg	1	9,46	VERIFIED	15/11/1996	15/11/1996			
223	Gnesta	1	3,19	VERIFIED	10/02/1999	10/02/1999			
224	Fresno	1	9,46	S2804/88FI	18/05/1995	18/05/1995			
225	Bonn	1	6,7						
226	clovelly	2	1,44	NCTC10436	08/07/1997	08/07/1997			
227	hofit	1	39						
228	chailey	1	6,8						
229	bulawayo	2	1,40	NCTC9948	24/08/1992	24/08/1992			
230	havana	1	13,23	S7035/92FH	18/05/1995	18/05/1995			
231	Kidderminster	1	38	VERIFIED	14/11/1995	14/11/1995			
232	kibusi	1	28						
233	thomasville	1	3,15,34	S4107/91FH	18/05/1995	18/05/1995			
234	okerara	1	3,10						
235	Tione	1	51	LASSWADE	08/01/1993	08/01/1993			
236	milwaukee	1	43	VERIFIED	15/11/1996	15/11/1996			
237	arizona	3	16	S7957/91AS	10/08/1992	10/08/1992			
238	3,15:-:-NM	1	3,15	S4218/91BC	18/04/1995	18/04/1995			
239	deversoir	1	45	VERIFIED	16/01/1997	16/01/1997			
240	babelsberg	1	28	LASSWADE	22/10/1992	22/10/1992			
241	taunton	1	18						
242	nottingham	1	16	S4256/91EO	18/05/1995	18/05/1995			
243	berkeley	1	43	VERIFIED	30/06/1995	30/06/1995			
244	dahlem	1	48	VERIFIED	19/09/1996	19/09/1996			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
245	brancaster	1	1,4,12,27						
246	arizona	3b	48	LASSWADE	10/08/1992	10/08/1992			
247	djakarta	1	48	VERIFIED	01/09/1995	01/09/1995			
248	arizona	3B	61	LASSWADE	09/04/1992	09/04/1992			
249	senegal	1	11						
250	ona	1	28	VERIFIED	06/11/1998	06/11/1998			
251	goldcoast	1	6,8	S5787/91FH	26/05/1995	26/05/1995			
252	new brunswick	1	3,15						
253	pietersburg	1	3,(15),(34)						
254	typhimurium var binn	1	rough						
255	weslaco	1	42	NCTC7411	05/10/1992	05/10/1992			
256	thompson var berlin	1	6,7						
257	Cannstatt	1	1,3,19	S13798/90FH	26/05/1995	26/05/1995			
258	Helsinki	2	1,4,12	S4018/91EO	23/04/1992	2/04/1992			
259	mara	1	39	LASSWADE	09/07/1996	09/07/1996			
260	berta	1	9,12	S6849/90BC	12/08/1992	12/08/1992			
261	foulpointe	2	38	VERIFIED	23/04/1992	23/04/1992			
262	arizona	3b	61	S5536/91SS	09/04/1992	09/04/1992			
263	budapest	1	1,4,12,27	LASSWADE	10/08/1993	10/08/1993			
264	Wayne	1	30	WHO89AK	23/10/1992	23/10/1992			
265	freemantle	2	42	LASSWADE	23/04/1992	23/04/1992			
266	Essen	1	4,12	S13141/90AS	26/05/1995	26/05/1995			
267	1,3,10:-:1,6	2	3,10	VERIFIED	23/04/1992	23/04/1992			
268	wassenaar	4	50	LASSWADE	27/03/1997	27/03/1997			
269	arkanass	1	3,15,34	S7739/91FH	26/05/1995	26/05/1995			
270	Makiso	1	6,7	LASSWADE	05/10/1993	05/10/1993			
271	1,9,12:-:1,5	1	1,9,12						
272	eimsbuettel	1	6,7,14	LASSWADE	23/09/1992	23/09/1992			
273	dar es sllaam	2	9,12	LASSWADE	23/04/1992	23/04/1992			
274	Portsmouth	1	3,15						
275	fischerkietz	1	1,6,14,25	S10122/91BT	09/07/1996	09/07/1996			
276	Marembe	1	44						
277	give	1	3,15	WHO1525/74	21/03/1997	21/03/1997			
278	manchester	1	6,8						
279	Caracus	1	(1)6,14(25)						
280	Salford	1	16						
281	arizona	3	38	S3042/91AS	12/05/92	12/05/92			
282	6,7:z29:-	2	6,7		22/04/1992	22/04/1992			
283	eves	1	6,14,24	NCTC6755	27/07/1993	27/07/1993			
284	rumford	1	6,7						
285	Morehead	1	30						

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
286	1,3,19:-:-NM	1	1,3,19						
287	35:-:-	1	35						
288	47:z4z23:-	1	47	S6946/91FH	26/05/1995	26/05/1995			
289	1,3,19:i:-	1	1,3,19						
290	9,46:z45:-	1	9,46	S4801/91FH	26/05/1995	26/05/1995			
291	3,10:-:-	1	3,10						
292	Deleted 23/11/98								
293	arizona	3	44	VERIFIED	22/04/1992	22/04/1992			
294	Illinois	1	3,15,34	S4020/94FH	22/05/1994	22/05/1994			
295	Teltow	1	28	S7270/92FH	12/05/1992	12/05/1992			
296	13,23:-:-NM	1	13,23						
297	London +ST104	1	3,10	WHO76K	20/05/1994	20/05/1994			
298	Luke	1	1,47						
299	eingedi	1	6,7	WHO2103/77	26/05/1995	26/05/1995			
300	Amherstiana	1	8	NCT6385	05/10/1992	05/10/1992			
301	Anfo	1	39	S6129/92FH	26/05/1995	26/05/1995			
302	sarajone	1	4,5,12						
303	Tamale	1	8,20						
304	kibi	1	16	S10624/91FH	19/05/1995	19/05/1995			
305	sendai(miami)	1	1,9,12	LASSWADE	10/08/1992	10/08/1992			
306	wuppertal	1	9,46	WHO1449/74	05/03/1997	05/03/1997			
307	Arizona	3b	11		09/04/1992	09/04/1992			
308	Arizona	3b	42						
309	freetown	1	38	S12450/91FH	19/05/1995	19/05/1995			
310	bispebjerg	1	(1)4(5)12						
311	glostrup	1	6,8	NCTC5757	01/07/1992	01/07/1992	17/06/1998		
312	plymouth	1	9,46						
313	ipswich	1	41						
314	matopeni	1	30	S15986/91BC	19/05/1995	19/05/1995			
315	Altona	1	8,20	S12550/91FH	19/05/1995	19/05/1995			
316	6,14,25:-:-NM	1	6,14,25						
317	typhi (Cat 3)		rough, Vi	NCTC8383					
318	nessziona	1	6,7	LASSWADE	10/08/1992	10/08/1992			
319	thielallee	1	6,7,14						
320	solt	1	11	S7665/92FH	09/01/1996	09/01/1996			
321	Chingola	1	11						
322	Papuana	1	6,7						
323	inganda	1	6,7						
324	pomona	1	28	S3291/92FH	19/05/1995	19/05/1995			
325	arizona	3a	41	S9117/92AS	09/04/1992	09/04/1992			
326	arizona	3b	61	LASSWADE	08/01/1993	08/01/1993			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
327	Putten	1	13,23	S10412/91FH	09/05/1995	09/05/1995			
328	Warral	1	42						
329	rubislaw	1	11	S3984/90MO	19/05/1995	19/05/1995			
330	selandia	1	3,15	WHO85K	04/03/1997	04/03/1997			
331	arizona	3b	16		09/04/1992	09/04/1992			
332	arizona	3a	44		07/04/1992	07/04/1992			
333	Arizona	3a	18	VERIFIED	03/09/1996	03/09/1996			
334	Senftenberg(R)	1	1,3,19	WHO2170/58	27/09/1994	27/09/1994			
335	berlin	1	17	LASSWADE	05/10/1992	05/10/1992			
336	Wandsworth	1	39						
337	halmstad	1	3,15						
338	omifisan	1	40	S395/89SS	19/05/1995	19/05/1995			
339	Millesi	1	1,40						
340	elizabethville	1	3,10	S10425/96BC	25/03/1997	25/03/1997			
341	chandans	1	11						
342	chameleon	4	16	LASSWADE	12/05/1992	12/05/1992			
343	Yeerongpilly	1	3,10						
344	muenster	1	3,10	S6688/91EO	19/05/1995	19/05/1995			
345	idikan	1	13,23	S4311/91FH	19/05/1995	19/05/1995			
346	Arizona	3b	65	WHO2420/78	05/03/1997	05/05/1997			
347	48:z29:-	4	48	VERIFIED	20/03/1998	20/03/1998			
348	massenya	1	(1)4,12(27)						
349	wildwood	1	3,15,34						
350	Bergen	1	47	S5814/91FH	19/05/1995	19/05/1995			
351	Toricado	1	42	S7657/91FH	19/05/1995	19/05/1995			
352	Minneapolis	1	3,15,34	S6136/92FH	19/05/1995	19/05/1995			
353	telelkebir	1	13,23	S5531/91SS	19/05/1995	19/05/1995			
354	nigeria	1	6,7						
355	arizona	3b	60						
356	Barranquilla	1	16						
357	tees	1	16	S7337/91/FH	19/05/1995	19/05/1995			
358	georgia	1	6,7	S4583/90FH	24/05/1995	24/05/1995			
359	simsbury	1	1,3,19	S4584/91FH	24/05/1995	24/05/1995			
360	58:d:z6	2	58	S5236/90AZ	24/05/1995	24/05/1995			
361	lanka	1	1,3,15	S5412/90FH	24/05/1995	24/05/1995			
362	Kokomlemle	1	39	S5688/90EW	24/05/1995	24/05/1995			
363	arizona	3	50		09/04/1992	09/04/1992			
364	jerusalem	1	6,7,14						
365	Widemarsh	1	35	S4125/97AS	13/06/1997	13/06/1997			
366	Ouakam	1	9,46	VERIFIED	11/09/1996	11/09/1996			
367	58:lz13:z6	2	58	S24047/91AS	22/04/1992	22/04/1992			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
368	Apapa	1	45	S183/91FH	24/05/1995	24/05/1995			
369	ituri	1	4,12	S1297/92FH	24/05/1995	24/05/1995			
370	ank	1	28	S15785/91FI	24/05/1995	24/05/1995			
371	karachi	1	45	S1587/92FI	24/05/1995	24/05/1995			
372	blegdam	1	9,12	NCTC5769	24/05/1995	24/05/1995			
373	madjorio	1	3,10	S1606/92FH	24/05/1995	24/05/1995			
374	Vogan	1	42	S3069/92FH	24/05/1995	24/05/1995			
375	Friedenau	1	13,22	S2140/92FH	24/05/1995	24/05/1995			
376	Kisarawe	1	11	S5077/92AS	26/05/1995	26/05/1995			
377	marina	4	48	S6440/89AS	08/05/1992	08/05/1992			
378	Moero	1	28	S5210/92FH	26/05/1995	26/05/1995			
379	Morningside	1	30	S6524/92FH	26/05/1995	26/05/1995			
380	agoueve	1	13,22	S6979/92HH	26/05/1995	26/05/1995			
381	javiana	1	1,9,12	NCTC6495	01/07/1992	01/07/1992			
382	onderstepoort	1	1,6,14,25	NCTC5795	01/07/1992	01/07/1992			
383	typhi murium + ST1	1	4,12	S7674/92PP	26/05/1995	26/05/1995			
384	paratyphi A (Cat.3)	1	1,2,12	NCTC0013					
385	dakar	1	28	NCTC9928	22/07/1992	22/07/1992			
386	Kirkee	1	17	NCTC5798	22/07/1992	22/07/1992			
387	minnesota	1	21	NCTC5800	22/07/1992	22/07/1992			
388	riogrande	1	40	NCTC7399	22/07/1992	22/07/1992			
389	Schlessheim	1	4,12,27	NCTC5732	22/07/1992	22/07/1992			
390	arizona	3	(6),14	S695/92AS	21/08/1992	21/08/1992			
391	arizona	3	51	S5692/92AS	21/08/1992	21/08/1992			
392	Mpouto	1	16	S9313/92AZ	26/05/1995	26/05/1995			
393	Rideau	1	1,3,19	S9488/92EO	26/05/1995	26/05/1995			
394	bredeney + ST6	1	1,4,12,27	SOMATIKIT	18/09/1992	18/09/1992			
395	Boecker	1	6,14,25	WHO89-65	05/11/1992	05/11/1992			
396	typhi (Cat.3)	1	9,12	NCTC8393					
397	Carrau	1	6,14,24	NCTC5794	19/01/1993	19/01/1993			
398	Arizona	3	65	S1507/93AS	31/05/1996	31/05/1996			
399	arechavaleta	1	4,5,12	NCTC6239	22/10/1992	22/10/1992			
400	Senftenberg(R)	1	3,19	NCTC10081	22/10/1992	22/10/1992			
401	Senftenberg(R)	1	3,19	S2185/94FI	20/04/1994	20/04/1994			
402	Mobeni	2	16	WHO1668-75	22/10/1992	22/10/1992			
403	Nitra	1	2,12	WHO193-66	23/10/1992	23/10/1992			
404	etterbeck	1	11	WHO1676K	23/10/1992	23/10/1992			
405	angoda	1	30	WHO767K	06/11/1992	06/11/1992			
406	Westerstede	1	3,19	WHO538-69	05/11/1992	05/11/1992			
407	Landau	1	30	WHO556K	05/11/1992	05/11/1992			
408	askraall	2	51	WHO1383-73	05/11/1992	05/11/1992			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
409	41:k:-	2	41	WHO105-65	05/11/1992	05/11/1992			
410	Congo	1	13,23	WHO1460K	26/05/1995	26/05/1995			
411	Senftenberg(R)	1	1,3,19	S14095/92FH	04/01/1993	04/01/1993			
412	houten	4	43	NCTC10401	08/01/1993	08/01/1993			
413	mondeor	2	39	WHO1232K	08/01/1993	08/01/1993			
414	53:lz28:z39	2	53	WHO1300K	25/06/1993	25/06/1993			
415	Montevideo var	1	6,7	WHO522/69	25/06/1993	25/06/1993			
416	Frankfurt	1	16	WHO1217K	25/06/1993	25/06/1993			
417	Harburg	1	(1),6,14,25	WHO962K	25/06/1993	25/06/1993			
418	lexington var	1	3,15,34	WHO133K	25/06/1993	25/06/1993			
419	40:z:z:42	2	40	WHO7531/91	25/06/1993	25/06/1993			
420	39:lv:1,5	2	39	WHO4053/83	25/06/1993	25/06/1993			
421	57:z29:z42	2	57	WHO1348K	25/06/1993	25/06/1993			
422	1,4,12,27:lv:z39	2	1,4,12,27	WHO1492/74	25/06/1993	25/06/1993			
423	28:r:enz15	2	28	WHO1617K	25/06/1993	25/06/1993			
424	42:r:- (nairobi)	2	42	WHO353K	25/06/1993	25/06/1993			
425	Infantis + ST30	1	6,7	S5757/95PH	25/03/1997	25/03/1997			
426	28:a:enx	2	2	WHO4059/83	25/06/1993	25/06/1993			
427	Arizona	3a	40	WHO1600/K	25/06/1993	25/06/1993			
428	43:z4z24:-	4	43	WHO1357/73	25/06/1993	25/06/1993			
429	Aderike	1	28	WHO1578/75	07/12/1993	07/12/1993			
430	crossness	1	67	WHO1422/K	16/11/1993	16/11/1993			
431	Arizona	3a	62	WHO5331/86	16/11/1993	16/11/1993			
432	Arizona	3a	63	WHOCDC5020	16/11/1993	16/11/1993			
433	43:gmst:z42	2	43	WHO1575/K	16/11/1993	16/11/1993			
434	Senftenberg(R)	1	(1),3,19	WHO1082/K	16/11/1993	16/11/1993			
435	Borreze	2	54	WHO2930/80	25/05/1994	25/05/1994			
436	60:z41:-	5	60	WHO4545/84	16/11/1993	16/11/1993			
437	1,9,12,46,27:c:z39	2	1,9,12,46,27	WHO877/K	16/11/1993	16/11/1993			
438	mountpleasant	1	47	WHO1245/K	16/11/1993	16/11/1993			
439	1,40,c:enxz15	2	(1),40	WHO1352/K	16/11/1993	16/11/1993			
440	wa	1	16	S2424/97CC	25/03/1997	25/03/1997			
441	66:z65:-	5	66	WHO2790/79	16/11/1993	16/11/1993			
442	4,12:-:enz15	1	4,12	WHOmono907	16/11/1993	16/11/1993			
443	kiel	1	2,12	WHO1017K	03/06/1994	03/06/1994			
444	Midway	1	6,14,24	WHO1818/76	03/06/1994	03/06/1994			
445	bahrenfeld	1	6,14,24	WHO1350K	03/06/1994	03/06/1994			
446	66:z41:-	5	66	WHO1224/72	28/07/1994	28/07/1994			
447	44:z4z24:-	4	44	S5513/94/EH	26/05/1995	26/05/1995			
448	koessen	1	2,12	WHO7067/89	09/12/1994	09/12/1994			
449	toricada	1	42	WHO653/69	09/12/1994	09/12/1994			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
450	riggil	1	6,7	WHO1447K	09/12/1994	09/12/1994			
451	yerba	1	54	WHO865-71	25/09/1995	25/09/1995			
452	Karamoja	1	40	WHO800K	09/12/1994	09/12/1994			
453	40:a:z39	2	40	WHO614K	09/12/1994	09/12/1994			
454	typhi (Cat.3)	1	9,12,Vi	WHO2864/79	25/09/1995	25/09/1995			
455	16:z:-	2	16	WHO1527/74	09/12/1994	09/12/1994			
456	43:d:z42	2	43		25/09/1995	25/09/1995			
457	Arizona	3a	41	WHO3064-61	25/09/1995	25/09/1995			
458	Arizona	3a	38	WHO594-54	26/09/1995	25/09/1995			
459	Arizona	3a	13,23		25/09/1995	25/09/1995			
460	senftenberg(R)	1	3,19	WHO1082K	25/09/1995	25/09/1995			
461	mikawasima(R)	1	6,7,(14)	WHO2547-60	26/09/1995	26/09/1995			
462	39:z48:-	1	39	WHO1344K	25/09/1995	25/09/1995			
463	Infantis (R)	1	6,7(14)	WHO1381K	25/03/1997	25/03/1997			
464	Arizona	3	42	WHO713/66	09/12/1994	09/12/1994			
465	6,8:a:z52	2	6,8	WHO1479K	25/09/1995	25/09/1995			
466	Arizona	3b	58	WHO5251-85	17/01/1997	17/01/1997			
467	Arizona	3b	38	WHO1699K	26/09/1995	26/09/1995			
468	Arizona	3b	43	WHO1213-72	25/09/1995	25/09/1995			
469	Arizona	3b	48	WHO556-59	25/09/1995	25/09/1995			
470	47:z58:-	1	47	WHO3188-81	25/09/1995	25/09/1995			
471	sternschanze (R)	1	30	WHO285-67	25/09/1995	25/09/1995			
472	Aesch	1	6,8	WHO1523-74	25/09/1995	25/09/1995			
473	4,12:z62:-	2	4,12	WHO277-67	16/01/1997	16/01/1997			
474	antarctica	1	9,12	WHO2145-77	26/09/1995	26/09/1995			
475	Aarrhus	1	18		25/09/1995	25/09/1995			
476	Arizona	3b	53	WHO2906-58	26/09/1995	26/09/1995			
477	13,23:z37:-	1	13,23	S7516/94/FH	14/10/1995	14/10/1995			
478	1,13,23:z37,z43:-	1	1,13,23	S5902/95/FH	19/07/1995	19/07/1995			
479	orientalis	1	16	S6993/95/AS	08/09/1995	08/09/1995			
480	Toucra	1	48	S6069/95/AS	14/11/1995	14/11/1995			
481	durban	1	9,12	S4820/94/AS	14/11/1995	14/11/1995			
482	sundsvall	1	6,14,25	S4398/94/AS	14/11/1995	14/11/1995			
483	vitkin	1	28	S5592/94/FH	14/11/1995	14/11/1995			
484	yoruba	1	16	S5403/94/FH	14/11/1995	14/11/1995			
485	patience	1	28	S4509/94/CC	14/11/1995	14/11/1995			
486	50:b:z6	2	50	S4472/94/AZ	14/11/1995	14/11/1995			
487	44:z4,z32:-	4	44	S3000/95/AS	14/11/1995	14/11/1995			
488	Freiburg	1	3,10	S2591/95/MO	14/11/1995	14/11/1995			
489	nchanga	1	3,10,(15)	S2851/95/FH	14/11/1995	14/11/1995			
490	Oakland	1	6,7	S2605/95/AS	14/11/1995	14/11/1995			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
491	nima	1	28	S2744/95/FI	14/11/1995	14/11/1995			
492	westhampton	1	3,10,(15,34)	S2884/95/FH	14/11/1995	14/11/1995			
493	stourbridge	1	6,8	S9290/95/CC	14/11/1995	14/11/1995			
494	newyork	1	13,22	S7768/94/EO	14/11/1995	14/11/1995			
495	42:z10:z6	2	42	S2273/95/AS	14/11/1995	14/11/1995			
496	Leiden	1	13,22	S2854/95/FH	14/11/1995	14/11/1995			
497	luckenwalde	1	28	S2768/95/FH	14/11/1995	14/11/1995			
498	Arizona	3	47	S9100/95/AS	14/11/1995	14/11/1995			
499	58:(lz13)lz28:z6	2	58	S7156/94/AS	21/11/1995	21/11/1995			
500	christiansborg	1	44	S5513/94/FH	23/11/1995	23/11/1995			
501	Arizona	3b	57	WHO2319/87	11/04/1997	11/04/1997			
502	cholerae suis	1	6,7	S12626/96AC	04/04/1997	04/04/1997			
503	uzaramo	1	6,14,25	NCTC8493	13/06/1997	13/06/1997			
504	altendorf	1	4,12	NCTC10546	17/06/1997	17/06/1997			
505	mgulani	1	38	NCTC8492	20/06/1997	20/06/1997			
506	enteritidis + ST10	1	9,12	S3379/97BC	24/06/1997	24/06/1997			
507	Dabou	1	8,20	WHO1441/74	20/08/1997	20/08/1997			
508	marshall	1	13,22	WHO1730/76	20/08/1997	20/08/1997			
509	naestved	1	1,9,12	WHO852K	20/08/1997	20/08/1997			
510	haelsingborn	1	6,7	WHO888K	20/08/1997	20/08/1997			
511	Oakland	1	6,7	WHO1592K	20/08/1997	20/08/1997			
512	Bulovka	1	6,7	WHO6693/88	20/08/1997	20/08/1997			
513	minnesota	1	21	WHO728K	20/08/1997	20/08/1997			
514	Sinthia	1	18	WHO1612K	20/08/1997	20/08/1997			
515	newport	1	6,8	WHO5038/85	20/08/1997	20/08/1997			
516	Havana	1	1,13,23	WHO6715/88	20/08/1997	20/08/1997			
517	pomona	1	28	WHO7411/90	20/08/1997	20/08/1997			
518	Hemingford	1	50	WHO7491/91	20/08/1997	20/08/1997			
519	Nyanza	1	11	WHO8199/94	20/08/1997	20/08/1997			
520	13,22:mt:z42:z39	2	13,22	WHO3405/81	14/08/1997	14/08/1997			
521	1,13,23:a:z42	2	1,13,23	WHO34/65	14/08/1997	14/08/1997			
522	13,23:k:z41	2	13,23	WHO2865/79	14/08/1997	14/08/1997			
523	17:mt:-	2	17	WHO1497/74	15/08/1997	15/08/1997			
524	53:lz28:z39	2	53	WHO1300K	14/08/1997	14/08/1997			
525	59:z:z6	2	59	WHO4045/83	14/08/1997	14/08/1997			
526	Arizona	3b	6,7	WHObeso/68	15/07/1997	15/07/1997			
527	Arizona	3b	17	WHOpc195	15/07/1997	15/07/1997			
528	Arizona	3b	42	WHO2101/77	31/07/1997	31/07/1997			
529	Arizona	3b	59	WHO3507/82	15/07/1997	15/07/1997			
530	Arizona	3b	38	WHO2224-56/	16/07/1997	16/07/1997			
531	Arizona	3b	40	WHO740-62/2	15/07/1997	15/07/1997			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
532	Arizona	3b	38	WHO7182/89	14/08/1997	14/08/1997			
533	Arizona	3b	58	WHO5250/85	15/07/1997	15/07/1997			
534	Arizona	3b	48	WHO5973/87	15/07/1997	15/07/1997			
535	Arizona	3b	58	WHO7095/89	14/08/1997	14/08/1997			
536	Arizona	3b	47	WHO5338/85	14/08/1997	14/08/1997			
537	Arizona	3b	48	WHO1476/74	15/07/1997	15/07/1997			
538	Arizona	3b	38	WHO3187/81	23/09/1997	23/09/1997			
539	66:z35	5	66	WHO1900/76					
540	66:z81	5	66	WHO7415/90					
541	48:z81	5	48	WHO4505/84	15/08/1997	15/08/1997			
542	50:lv:z67	6	50	WHO8364/95	14/08/1997	14/08/1997			
543	paratyphi B (Cat.3)	1	4,5,12	NCTC10412	08/07/1997	08/07/1997			
544	bruck	1	6,7	S4270/97/BC	19/08/1997	19/08/1997			
545	noya	1	8	S4973/97/CC	19/08/1997	19/08/1997			
546	Llandoff	1	1,3,19	RIVM1169595	23/09/1997	23/09/1997			
547	Give	1	3,10	RIVM1169695	23/09/1997	23/09/1997			
548	Virchow	1	6,7	RIVM1169795	23/09/1997	23/09/1997			
549	oranienburg	1	6,7	RIVM1169895	23/09/1997	23/09/1997			
550	47:d:z39	2	47	RIVM1169995	23/09/1997	23/09/1997			
551	enteritidis	1	9,12	RIVM1170095	23/09/1997	23/09/1997			
552	lille	1	6,7	RIVM1170195	23/09/1997	23/09/1997			
553	berta	1	9,12	RIVM1170395	23/09/1997	23/09/1997			
554	Typhimurium	1	4,5,12	RIVM1170495	23/09/1997	23/09/1997			
555	Poona	1	13,22	RIVM1170595	23/09/1997	23/09/1997			
556	16:z4z32:-	4	16	RIVM1170795	23/09/1997	23/09/1997			
557	Dublin	1	9,12	RIVM1170895	23/09/1997	23/09/1997			
558	Infantis	1	6,7	RIVM1170995	23/09/1997	23/09/1997			
559	worthington	1	13,23	RIVM1171095	23/09/1997	23/09/1997			
560	Fluntern	1	(6)18	RIVM1171195	23/09/1997	23/09/1997			
561	kentucky	1	8,20	RIVM1171295	23/09/1997	23/09/1997			
562	Agona	1	4,12	RIVM1171495	23/09/1997	23/09/1997			
563	shubra	1	4,5,12	S7474/EO/97	10/10/1997	10/10/1997			
564	Arizona	3	47	S3349/96/AS	15/10/1997	15/10/1997			
565	agoueve	1	13,22	S3892/96/AS	15/10/1997	15/10/1997			
566	Arizona	3	48	S10432/95/AS	15/10/1997	15/10/1997			
567	Arizona	3	50	S3340/96/AZ	15/10/1997	15/10/1997			
568	c.suis v. kunzendorf	1	6,7	NCTC5737	19/11/1997	19/11/1997			
569	california	1	4,12	NCTC6018 55	19/11/1997	19/11/1997			
570	Dublin	1	9,12	Replace	19/11/1997	19/11/1997			
571	typhimurium	1	4,5,12	Replace	19/11/1997	19/11/1997			
572	gallinarum	1	9,12	NCTC423 180	19/11/1997	19/11/1997			

ST.NO	SEROTYPE	SG	SOMATIC	SPECIES	MASTER SET	WORKING SET 1	WORKING SET 2	WORKING SET 3	WORKING SET 4
573	pullorum	1	9,12	NCTC5776971	19/11/1997	19/11/1997			
574	Haduna	1	4,12	S10256/97AD	09/12/1997	09/12/1997			
575	aberdeen	1	11	NCTC5791	08/01/1998	08/01/1998			
576	Haduna	1	4,12	S10255/97AD	24/03/1998	24/03/1998			
577	llobregat	1	44	S10838/97AD	24/03/1998	24/03/1998			
578	mississippi	1	13,23	S11032/97AD	24/03/1998	24/03/1998			
579	tamberma	1	047?	S763/98AD	24/03/1998	24/03/1998			
580	Lansing	1	38	S2035/98AD	24/03/1998	24/03/1998			
581	Cotham	1	38	S2310/93AD	24/03/1998	24/03/1998			
582	paratyphi A (Cat.3)	1	1,2,12	NCTC8002					
583	typhimurium	1	4,5,12	D3389-1/92	03/09/1998	03/09/1998			
584	c-suis v.kunzendorf	1	6,7	DVL143	03/09/1998	03/09/1998			
585	typhimurium	1	4,5,12	S153/98PP	14/10/1998	14/10/1998			
586	Infantis	1	6,7	S1495/96PP	14/10/1998	14/10/1998			
587	derby	1	4,12	S74/98PP	14/10/1998	14/10/1998			
588	goldcoast	1	6,8	S6239/98PP	14/10/1998	14/10/1998			
589	panama	1	9,12	S2649/98PP	14/10/1998	14/10/1998			
590	kedougou	1	13,23	S2801/98PP	14/10/1998	14/10/1998			
591	Anatum	1	3,10	S6236/98PP	14/10/1998	14/10/1998			
592	Infantis	1	6,7	S6285/95PP	21/01/1999	21/01/1999			
593	Derby	1	4,12	S152/98PP	21/01/1999	21/10/1999			
594	panama	1	9,12	S94/98PP	21/01/1999	21/10/1999			
595	kegougou	1	13,23	S823/98PP	21/01/1999	21/10/1999			
596	Anatum	1	3,10	S8426/97PP	21/01/1999	21/10/1999			
597	Infantis	1	6,7	57432217/93	24/01/1999	21/01/1999			

Reference
Cryopreservation of Fungal Spores using Porous Beads (Microbank™)

D. CHANDLER

Horticulture Research International, Wellesbourne, Warwick. CV35 9EF UK

A simple method is described for the cryopreservation of conidia, stored adhering to small porous beads in a robust polypropylene vial. For recovery, a single bead is removed from the vial and streaked onto a solid growth medium. Preparative work is minimised so the method is rapid. The storage life of an isolate can be increased greatly if a 'seed lot' system is employed.

The most reliable way to store fungi for extended periods is in liquid nitrogen or in the vapour of liquid nitrogen. Elliott (1976) used polypropylene drinking straws to store strains of *Agaricus bisporus* in liquid nitrogen as the straws were safer and less expensive than using glass ampoules. The small size of the straws also permitted greater storage capacity and sample replication in vivostats. However, there are problems with drinking straws as their small size makes them difficult to handle, they are hard to check for leaks after sealing and they can burst when removed from a vivostat. The preparative work is also time-consuming, so cultures tend to be handled in batches which increases the chances of cross contamination. Stalpers, De Hoog and Vlug (1987) recommended the use of special apparatus for large scale application of the straw technique but this apparatus is not available commercially.

This note describes cryopreservation of conidia of mitotic, entomopathogenic fungi on porous beads. The 'Microbank™' storage system (Pro-Lab Diagnostics, Bromborough, Wirral UK, L62 3PW) is a commercial variant of a system designed originally for storing bacteria (Feltham *et al*, 1978) but also appears suitable for storing certain fungi. In our laboratory at Wellesbourne, cultures are stored in the vapour above liquid nitrogen, but porous beads could be used for storing microorganisms in a deep freeze (ca -70°C). The 'Microbank™' system consists of sterile vials containing beads (3 mm diam.) which act as carriers to support the microorganisms. Each vial has a volume of 2 ml and contains 25 beads in 1 ml of a cryopreservative (usually 10 or 15% glycerol). As the 'Microbank™' system is available commercially, preparative work is kept to a minimum. Vials and beads are available in a range of colours to aid identification and vials are robust and easy to handle.

If desired, however, beads and the vials can be obtained separately from many laboratory suppliers.

In this laboratory, the preparation, inoculation and recovery of conidia are performed in a microbiological safety cabinet. A suspension of conidia, prepared in sterile 0.05% Tritron X-100 surfactant, is placed in a sterile Eppendorf tube, centrifuged, washed and centrifuged again. The pellet is then resuspended in cryopreservative taken from one of the vials and pipetted back into that vial, which is inverted 3 to 4 times to allow conidia to adhere to the beads. Most of the cryopreservative is then removed to prevent the beads sticking together during freezing, but a thin layer of free liquid can be left at the bottom of the vial to allow recovery of the fungus should the conidia fail to adhere to the beads. Vials are frozen overnight in a deep-freeze at -70° and transferred to a vivostat the following morning. The vials are stored in the vapour above liquid nitrogen as this avoids the problem of liquid nitrogen entering the vials through cracks or leaks.

For recovery, vials are removed from the vivostat and one bead is removed with sterilised forceps and streaked, using a loop, onto the surface of a suitable solid medium. Vials are prevented from thawing during this procedure by placing them into an insulated block of aluminium (10 x 8 x 4 cm) or 'Cryoblock', which has sample wells drilled into it. The block is stored in the deep freeze and can be cooled further in liquid nitrogen immediately before use. The method is simple and rapid so that the vials are out of the vivostat for only two to three minutes. The temperature of a vial placed within the cryoblock was measured on the laboratory bench at room temperature (approximately 20°) using a thermocouple, after they had been frozen overnight at -70°. Consistently, the cryoblock kept the vial at a temperature below -60°for five min. below -50° for 15 min and below -40° for 30 min.

The method has been used to store approximately 50 isolates of *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii* and *Paecilomyces* spp. *Prima facie,* the method appears to be suitable only for storing conidia: it remains tl be seen whether it is also suitable for preserving other life stages, e.g. hyphal bodies produced in liquid culture. To date, conidia have been stored for 18 months, with recovery of a bead every 3 months, so that the suitability of the method for very long term preservation has not been assessed. However, all isolates stored in this way have been recovered without contamination and loss of pathogenicity to target insects has not been observed in our routine bioassays. A preliminary assessment of the germination of conidia was performed for two isolates each of *B. bassiana, M. anisopliae* and *V. lecanii.* Each isolate, obtained from cultures stored previously for a minimum of six years in polypropylene straws under liquid nitrogen, had been stored for 18 months using the bead system. Conidia were washed from beads in 1 ml 0.05% Triton X-100 and an aliquot (0.1 ml) pipetted onto Sabouraud dextrose agar in 5.5 cm Petri dishes. Dishes were incubated at 23° for 24 hr, after which conidia were stained with lactophenol cotton blue and germinated/ungerminated conidia were counted using a compound

miscroscope. Conidia were considered to have germinated when the length of the germ tube exceeded the width of the conidium. Three replicates were used with a minimum of 300 conidia counted each time. Germination of conidia was greater than 95% in all cases. Each bead held between 10^5 and 10^6 conidia.

An isolate can be stored in a 'seed lot' system to increase its storage life. Two vials are prepared from the isolate. One vial, the 'working' vial, is used for preparing cultures for experimental work. When all the beads from the working vial have been used, a bead from the other 'seed' vial is removed, a culture is grown from it and is used to prepare a second working vial. In this laboratory, a further level of storage has been added. A slope culture from the working vial is kept in a refrigerator at 5° for 3 months and cultures for experiments are grown from this. As each working vial contains 25 beads, it should last for over 6 years. Theoretically, therefore, an isolate stored using this method of cryopreservation can be accessed for 156 years before it needs to be replaced.

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Feltham, R.K.A, Power A.K., Pell P. A. & Sneath, P.H.A (1978). A simple method for storage of bacteria at -76º C. *Journal of Applied Bacteriology 44. 313-316.*
Stalpers, J.A., De Hoog A & Vkyg I. J. (1987). Improvement of the straw technique for the preservation of fungi in liquid nitrogen. *Mycologia 79. 82-89.*

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Reference

DATA ON STORAGE OF NCTC REFERENCE SET OF CULTURES SELECTED FOR QUALITY CONTROL AND LABORATORY ACCREDITATION REQUIREMENT USING MICROBANK™

Introduction

Ashford PHL carried out an investigation using cultures supplied by NCTC to select cultures suitable for use in QC procedures in Food, Water and Dairy products testing. These were initially directed to Public Health Laboratory's requirements but were extended to cover other Laboratories' requirements.

Following feedback from colleagues, both in the PHLS and other sources, a set of 44 cultures were selected.

Method

The freeze dried vials received from NCTC were checked for vacuum integrity using a spark tester. Vials were then opened following the NCTC instructions under a Class III safety cabinet. The hydrated cultures were then inoculated into appropriate media (in most cases Blood Agar). The incubation temperature, gaseous requirement and length of incubation were selected as appropriate to the culture eg 37°C aerobic 24 hours fore most cultures.

The incubated cultures were examined for viability and purity. A subculture was prepared for identification check as appropriate to the individual culture.

Inoculation of the Vials and Beads

Using the primary culture obtained directly from the NCTC vial the vials were inoculated following the ProLab Procedure A Preparation.

Storage

The vials were placed in a -70°C Cabinet (Kelvinator).

Check of Viability of Beads

All inoculated beads were checked for viability within 1 week of inoculation, all were viable.

Recovery Method

The Pro-Lab Procedure B for recovery was followed. In addition "cold blocks" were used to carry the culture from the -70°C Cabinet and during testing. Recovery was limited to 6 cultures at a time to reduce temperature loss.

The beads were inoculated onto the appropriate media and appropriate culture conditions were used. Recovery was assessed by a semi-quantitative scoring method.

Recovery Results

The dates of bead inoculation and last date of viability (recovery) check are given on the enclosed list.

All cultures were viable at the recent dates shown. The majority have also been tested previously. All cultures remained viable with no quantitative loss of viability detected.

The majority of the cultures have been stored on beads at -70°C for over 2 years with no loss of viability. Tests for storage at other temperatures e.g. -20°C were not performed.

Conclusion

Storage of the NCTC Reference Set of Cultures has been demonstrated under normal working conditions in a Public Health Laboratory using ProLab Microbank™ system.

This method of storage of cultures is recommended for use in microbiological Laboratories requiring reference cultures for Laboratory Accreditation and QC procedures.

The NCTC set of cultures is recommended for this purpose.

Terence J Donovan PhD
Environmental Microbiologist
Ashford Public Health Laboratory

Results

1.	Zygosaccharomyces rouxii	NCPF 3879	(26)
2.	Aspergillus niger	NCPF 2275	(26)
3.	Bacillus cereus	NCTC 7464	(26)
4.	Bacillus subtilis	NCTC 10400	(26)
5.	Campylobacter jejuni (BT1)	NCTC 11322	(26)
6.	Saccharomyces cerevisiae	NCTC 3178	(26)
7.	Citrobacter freundii	NCTC 9750	(27)
8.	Clostridium difficile	NCTC 11204	(26)
9.	Clostridium perfringens	NCTC 8237	(26)
10.	Enterobacter aerogenes	NCTC 10006	(27)
11.	Enterococcus faecalis	NCTC 775	(27)
12.	Escherichia coli	NCTC 10418	(9)
13.	Escherichia coli 0157:H7	NCTC 12079	(26)
14.	Lactobacillus casei	NCTC 10302	(17)
15.	Legionella pneumophila SG 1	NCTC 12821	(27)
16.	Listeria monocytogenes 4b	NCTC 11994	(26)
17.	Proteus mirabilis	NCTC 10975	(26)
18.	Pseudomonas fluorescens	NCTC 10038	(24)
19.	Pseudomonas aerugionosa	NCTC 10662	(26)
20.	Pseudomonas cepacia	NCTC 10661	(26)
21.	Salmonella typhimurium	NCTC 12023	(26)
22.	Shigella sonnei	NCTC 8574	(26)
23.	Staphylococcus aureus	NCTC 6571	(26)
24.	Staphylococcus epidermidis	NCTC 11047	(20)
25.	Lactobacillus lactis	NCTC 662	(26)
26.	Listeria ivanovii	NCTC 11846	(24)
27.	Campylobacter coli	NCTC 11366	(25)
28.	Vibrio furnissii	NCTC 11218	(26)
29.	Vibrio parahaemolyticus	NCTC 10885	(26)
30.	Yersinia enterocoliticus SG1	NCTC 10460	(26)
31.	Klebsiella aerogenes	NCTC 9528	(26)
32.	Aeromonas hydrophila	NCTC 8049	(26)
33.	Acinetobacter lwoffii	NCTC 5866	(26)
34.	Serratia marcescens	NCTC 11935	(26)
35.	Edwardsiella tarda	NCTC 11934	(26)
36.	Protes rettgeri	NCTC 7475	(26)
37.	Enterobacter cloacae	NCTC 11936	(26)
38.	Vibrio cholerae Non 0:1/0:24	NCTC 11348	(26)
39.	Salmonella Poona 013, 22	NCTC 4840	(26)
40.	Rhodococcus equii	NCTC 1621	(26)
41.	Staphylococcus aureus	NCTC 1803	(26)
42.	Clostridium bifermentans	NCTC 506	(26)
43.	Clostridium sporogenes	NCTC 532	(26)
44.	Listeria innocua	NCTC 11288	(26)

Reference
Validation for Cryo Storage of *Brucella* spp.using Microbank™

Rationale

The *Brucella* research section of the Veterinary Laboratories Agency (VLA) under the auspices of the Food and Agriculture Organisation (FAO)/World Health Organisation (WHO) Collaborating Centre for Reference and Research on Brucellosis and on behalf of the Office International des Epizooties (OIE) Brucellosis Reference Centre, has as one of our roles to advise other laboratories on the identification and preservation of *Brucella* strains. As finances for these laboratories can be a problem it is not always possible to freeze-dry samples. On a recent VLA workshop in Morocco the delegates from North African countries were presented with Microbank™ cryo vials as an alternative storage method. They thought that these were ideal for their needs. We would like to be in a position to recommend these vials to colleagues at other laboratories worldwide with confidence of their suitability for the long-term storage of *Brucella.*

Materials and Methods

A representative isolate of each sub-species and in addition the most fastidious strain of *Brucella abortus* biovar 2 (Advances in Brucellosis Research, Texas A & M University Press, Texas) henceforth referred to as a set, were sub-cultured onto serum dextrose agar (SDA) to check for purity. Each reference strain was freeze dried allowing one vial for each date of testing in the trial, they were also added to the Microbank™ cryo vials in accordance with the manufacturers instructions. In order to ascertain the effects of freeze/thawing an individual vial was set up for each of the storage conditions and each testing date. After one month of storage at +4°C the first set of freeze dried vials were reconstituted and subbed onto SDA plates to give a circle of approximately 5cm in diameter. The Microbank™ cryo vials from the -20°C and -80°C freezers, a bead was removed from these and spread on SDA plates to give a circle approximately 5cm in diameter, all plates were then incubated at 37°C in a 10% CO_2 atmosphere for 4 days. Growth was examined for morphology and the quantity was compared allowing a deviation of approximately 25% growth between the cultures of different storage conditions. Although the inoculum was not standardised, it is important that sufficient quantity of the isolate remains viable in order to carry out further work on the isolates. The morphology was examined visually, aided using obliquely reflected light from under the culture.

After six months this was repeated, however this time beads were removed from the original Microbank™ cryo vials, opened at one month and an additional new bead from a fresh vial was removed for the sixth month stage in order to assess the effect of freeze/thawing. This process was also repeated after one year of storage.

Results

The results for the first year of this trial are as follows:

Table Key:

++	Growth comparable to freeze-dried vials
+-	Growth, but insufficient to be comparable to freeze drying
+	Freeze dried vials growth (used as the standard for comparison against)
-	No growth
?	Change in morphology of the culture
!	Contaminant present

Freeze-dried vials stored at +4°C

Isolate	1 month vial	6 month vial	1 year vial
Brucella melitensis (biovar 1) 16M	+	+	+
Brucella abortus (biovar 1) 544	+	+	+
Brucella suis (biovar 1) 1330	+	+	+
Brucella canis RM6/66	+	+	+
Brucella neotomae 5K33	+	+	+
Brucella abortus (biovar 2) 86/8/59	+	+	+
Brucella ovis 63/290	+	+	+

Validation for Cryo Storage of *Brucella* spp (Cont'd)

Microbank™ cryo vials stored at -20°C

Isolate	1 month vial	6 month vial	1 year vial	Time in storage
Brucella melitensis (biovar 1) 16M	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella abortus (biovar 1) 544	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella suis (biovar 1) 1330	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella canis RM6/66	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella neotomae 5K33	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella abortus (biovar 2) 86/8/59	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella ovis 63/290	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year

Validation for Cryo Storage of *Brucella* spp (Cont'd)

Microbank™ cryo vials stored at -80°C

Isolate	1 month vial	6 month vial	1 year vial	Time in storage
Brucella melitensis (biovar 1) 16M	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella abortus (biovar 1) 544	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella suis (biovar 1) 1330	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella canis RM6/66	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella neotomae 5K33	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella abortus (biovar 2) 86/8/59	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year
Brucella ovis 63/290	++	++	++	1 month
	++	++	++	6 months
	++	++	++	1 year

Conclusion

The preliminary results have so far shown that the procedure could be a cost effective alternative to freeze-drying negating the problems associated with freeze storage of cultures, such as loss of viable organisms through lysis during freeze thawing owing to the formation of ice crystals. The yield and morphology of the Microbank™ cryo vials in this first year have proved to be comparable with that of freeze-drying. All the cultures were still viable after the first year of this trial and as of yet there has been no significant difference in the products stored at -20°C and those stored at -80°C. **This study has also shown that the freeze thawing of the Microbank™ cryo vials has limited effect so far, proving the efficacy of the cryopreservative.**

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Reference
Long-Term Preservation of Fungal Isolates in Commercially Prepared Cryogenic Microbank™ Vials

A. Espinel-Ingroff,[1]* D. Montero,[2] and E. Martin-Mazuelos[2]
VCU Medical Centre, Richmond, Virginia[1] and Valme University Hospital, Seville, Spain[2]

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Since 1994, 6,198 yeasts and 391 moulds belonging to 25 and 37 species, respectively, were stored in Microbank™ cryogenic vials at ≥ -130°C in liquid nitrogen and at -70°C in a freezer. All of the isolates, with the exception of 45 yeasts and 15 dermatophytes, were recovered from both storage temperatures. Good reproducibility was demonstrated for amphotericin B, fluconazole and voriconazole MICs determined for random isolates.

Long-term preservation of fungal strains is essential for their in-depth study; however, both the viability and the stability of living cells should be ensured during the preservation period. Fungal isolates are usually preserved in water at room temperature (10), an easy and economical procedure introduced for fungi by Castellani in 1939 (5). Because the stability of fungal cells was not ensured by this simple procedure, other methods have been suggested, such as preservation in soil or on oil or water-covered slants, cryopreservation either in liquid nitrogent or at low temperature (-20 and -70°C) (2-5, 7, 9-11, 14, 16), and lyophilization (the freeze-drying procedure) (1, 15). Cryopreservation in liquid nitrogen and lyophilization are the methods recommended and used by the American Type Culture Collection (1). Although lyophilization of living cells provides a mechanism for stabilizing these cells for long periods of time, this procedure is cumbersome and lengthy and requires expensive equipment. On the other hand, storage in liquid nitrogen vapour (above the liquid at ≥ -130°C) is a more convenient and less expensive alternative for long-term storage of living cells. Storage above the liquid nitrogen prevents leakage of the liquid nitrogen into the vials.

The Microbank™ system (Pro-Lab Diagnostics, Texas, USA) consists of sterile vials that contain 25 porous, coloured beads and a cryopreservative fluid; this system was originally developed for storage of bacterial cells (8). The beads are acid washed, and their porous nature allows the cells to adhere to the bead surface; the beads serve as carriers for the cells being

stored (Microbank™ package insert). When an isolate is stored in this way, 25 or more identical cultures can be preserved. The purpose of this study was to evaluate the preservation in Microbank™ sterile vials of yeast and mould clinical isolates that were received from 1994 to the end of 2002 at the VCU Medical Center (Richmond, Va.) and the Valme University Hospital (Seville, Spain). Two temperatures (≤ -130°C {liquid nitrogen vapour} and -70°C {freezer}) were evaluated.

*Corresponding author. Mailing address: Division of Infectious Diseases, Medical Mycology Research Laboratory, VCU Medical Centre, 1101 E.Marshall St., P.O. Box 980049, Richmond, VA 23298-0049. Phone: (804) 828-9711. Fax: (804) 828-3097. E-mail: avingrof@hsc.vcu.edu

Fresh, pure cultures of 6,198 yeast and yeast-like organisms and 391 moulds - **(Table 1)** were grown on either Sabouraud dextrose agar (for yeasts) or potato dextrose agar (for moulds) at 35°C; some isolates of dermatophytes, *Histoplasma capsulatum, Blastomyces dermatitidis* and *Alternaria* spp. were incubated at 30°C. Yeast and Yeast-like isolates were incubated for 48 to 72 hours and moulds were incubated for 7 to 15 days. Each isolate was stored in accordance with the directions of the manufacturer. For each isolate, the cryogenic fluid of two Microbank™ vials was inoculated with the fungal growth to a density approximately equivalent to a McFarland standard of 3 or 4. The inoculated fluid was mixed four or five times to emulsify the suspension and to bind the cells to the beads. The extraneous cryogenic fluid was then removed, leaving the inoculated beads as free of liquid as possible to prevent the beads from sticking together during freezing but allowing a thin layer of suspension to stay at the bottom of the vial. The vials were then held overnight at -70°C. After overnight freezing, one of the vials was stored in liquid nitrogen vapour (≤ -130°C) and the other was left at -70°C.

The viability and purity of the strains were monitored immediately after storage, at 1 and 6 months after storage, and once a year subsequently as follows. One of the inoculated beads was removed under aseptic conditions with a sterile needle, and each vial was returned immediately to the corresponding low temperature; the bead was then inoculated onto either Sabouraud dextrose agar or potato dextrose agar for at lease 20 days. Both viability and the morphological characteristics of each culture were observed.

Each mould isolate was considered viable if the rate of growth present was the same as that of the original culture and if the morphology and colour of the colony matched the fungal identification documented for each strain. All of the mould strains, with the exception of 15 (of 61) dermatophyte isolates, were recovered each time from both storage temperatures and showed the initial colony characteristics, growth rates, and morphologies **(Table 1)**. Only isolates of *B. dermatitidis, H. capsulatum*, and *Alternaria* spp. required more than one bead for harvesting; they required two to four beads. These results are in agreement with those described by Chandler (6), who preserved 50

uncommon moulds for 18 months in Microbank™ vials and found that only one bead was necessary for the recovery of most isolates. Each yeast strain was considered viable if growth was present; the identification and purity of yeasts were also randomly validated on CHROMagar medium. A very small percentage of yeasts (0.7%) were not recovered; *Candida dubliniensis* had the lowest recovery rate (33%; 28 of the 42 isolates were not recovered). The stability was validated by determining the antifungal susceptibilities of random samples of yeasts (200 isolates) and moulds (50 isolates) stored at both temperatures. Amphotericin B, fluconazole and voriconazole MICs were determined by following NCCLS guidelines (documents M27-A2 {for yeasts} and M38-A {for moulds} (12, 13) before storage and 6 months and 4 years after preservation. MICs for the isolates after storage were either the same as, or within three dilutions of the MICs before storage, which is the criterion used in NCCLS studies to obtain percentages of intra-and inter-laboratory reproducibilities as well as for establishing quality control MIC ranges (12, 13).

In general, the effects of both storage temperatures on the stability and viability of stored isolates were similar, which is fortunate because most laboratories have a
-70°C freezer.

The advantage of using the Microbank™ system over other cryogenic systems (4, 7, 9, 11, 14, 16) is its commercial availability. The time-consuming procedure of preparing other preservative devices such as drinking straws (16) or cryogenic fluid is avoided; Microbank™ vials are stored at room temperature prior to use. The harvesting of individual isolates is easier than that described by Pasarell and McGinnis (14), in which a portion of the frozen culture is chipped and sub-cultured. Because vials should not be outside the low-temperature device for more than 3 minutes to avoid thawing, it is recommended that the frozen vials be placed in an insulated cryoblock during harvesting.

In conclusion, the Microbank™ system appears to be an easy, convenient, economical and effective tool for the preservation of fungal isolates other than dermatophyte and *C.dubliniensis* strains. Longer monitoring of isolates and storage of other species would further validate the reliability of this system for the cryogenic preservation of yeast and mould strains. Also, the stability of fungal cells should be further assessed by molecular parameters.

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TABLE 1 – Fungal isolates reserved between 1994 and 2002

Species	No. of isolates stored	No. (%) of isolates not recovered
Yeasts and yeast-like organisms:	4,453	5
Candida albicans	5	0
Candida ciferri	42	28
Candida dubliniensis	28	0
Candida famata	359	0
Candida glabrata	33	0
Candida guilliermondii	6	0
Candida humicola	10	0
Candida kefyr	279	5
Candida krusei	20	0
Candida lambica	5	0
Candida lipolytica	43	0
Candida lusitaniae	352	3
Candida parapsilosis	3	0
Candida rugosa	401	2
Candida tropicalis	14	0
Candida zeylanoides	112	1
Cryptococcus neoformans	3	0
Cryptococcus albidus	9	0
Cryptococcus laurentii	4	0
Hansenula anomala	1	0
Sporobolomyces salmonicolor	2	0
Trichosporon beigelii	6	1
Rhodotorula rubra	1	0
Rhodotrula glutinis	7	0
Saccharomyces cerevisiae		
TOTAL YEAST AND YEAST-LIKE ORGANISMS:	**6,198**	**45 (0.7)**
Moniliaceous moulds:		
Aspergillus fischeri	1	0
Aspergillus flavus	24	0
Aspergillus fumigatus	117	0
Aspergillus niger	10	0
Aspergillus nidulans	9	0
Aspergillus terreus	27	0
Aspergillus sydowii	1	0
Aspergillus versicolor	1	0
Fusarium incarnatum	1	0
Fusarium moniliforme	5	0
Fusarium solani	10	0
Fusarium oxysporum	5	0
Paecilomyces lilacinus	9	0
Rhizopus arrhizus	7	0
Rhizomucor pusillus	1	0
Trichoderma longibrachiatum	4	0
TOTAL MONILIACEOUS MOULDS:	**232**	**0**

TABLE 1 – Fungal isolates reserved between 1994 and 2002 (Cont'd)

Species	No. of isolates stored	No. (%) of isolates not recovered
TOTAL YEAST AND YEAST-LIKE ORGANISMS – B/F	**6,198**	**45 (0.7)**
TOTAL MONILIACEOUS MOULDS – B/F	**232**	**0**
Dematiaceous moulds:		
Alternaria spp.	4	0
Bipolaris hawaiiensis	3	0
Bipolaris spicifera	3	0
Cladophialophora bantiana	8	0
Cladosporium cladosporioides	1	0
Curvularia spp.	4	0
Dactylaria gallopava	3	0
Exophiala jeanselmei	6	0
Exophiala spinifera	2	0
Phoma sp.	1	0
Scedosporium apiospermum	17	0
Scedosporium prolificans	10	0
Wangiella dermatitidis	9	0
Dimorphic moulds:		
Blastomyces dermatitidis	5	0
Histoplasma capsulatum	5	0
Penicillium marneffei	17	0
Dermatophytes:		
Epidermophyton floccosum	4	2
Microsporum canis	12	3
Microsporum gyseum	6	1
Trichophyton mentagrophytes	26	2
Trichophyton rubrum	13	7
TOTAL MOULDS:	**391**	**15**

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Use of Commercially Available Cryogenic Vials for Long-Term Preservation of Dermatophyte Fungi

M. Baker[1] and P. Jeffries[2]*

East Kent Microbiology Service, The William Harvey Hospital, Kennington Road, Ashford, Kent TN24 0LZ,[1] Department of Biosciences, University of Kent, Canterbury, Kent CT2 6NJ, United Kingdom[2]

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The use of commercially available cryogenic vials (Microbank vials) stored at –70°C for the storage and preservation of dermatophyte fungi was investigated. None of the 200 strains of dermatophytes examined, representing 21 species, showed a loss of viability after they had been stored for periods ranging from 1 week to 2 years at –70°C. All strains showed typical colonial and microscopic morphologies following revival.

Long-term storage of fungal isolates is critical for preservation of the germplasm and maintenance of stock cultures with minimal effort over long periods. Conservation of morphological, physiological, genetic, and metabolic stability is crucial for many purposes and is vital for isolates used as medical reference strains, for chemotaxonomic studies, or in the commercial production of biochemicals. In a comparative study (**3**) of the effects of five different storage methods, cryopreservation was the method that best provided for the stability of secondary metabolite production and is now considered the best method available for the long-term storage of microbial cultures (**4**). Dermatophytic fungi can present problems for storage, as the cultures often become pleomorphic, with various levels of sporulation or mycelial growth. McGinnis et al. (**2**) used sterile distilled water to store hyphal and spore suspensions of 147 different species of fungi at 25°C for periods of 12 to 60 months. These included more than 25 species of dermatophytes. The degree of sporulation and the quality of the inoculum appeared to be critical factors; and when the inocula were "adequate" in size, even some poorly sporulating species such as *Trichophyton violaceum*, *Trichophyton schoenleinii*, and *Microsporum ferrugineum* survived storage well. The long-term storage of a wide range of fungal species, including dermatophyte isolates, in commercially prepared cryogenic freezer beads (Microbank) at –70°C or in liquid nitrogen has been tested (**1**). Although most fungi were preserved well by this method, dermatophytic fungi did not show good recovery rates. For example, more than 50% of isolates of *Trichophyton rubrum* were not recovered. Consequently, this method was not recommended for use for the long-term storage of dermatophytic fungi. In contrast, we have found contradictory results and we report on a simple and successful technique for the long-term storage of dermatophytes.

Fresh isolates were collected from clinical specimens submitted to the Microbiology Department at the William Harvey Hospital, Ashford, United Kingdom, between March 2002 and August 2005. Reference strains were obtained from the National Collection of Pathogenic Fungi, Bristol, United Kingdom. Representative strains of dermatophytes were used to assess a commercially available freezer bead storage kit (Microbank; Pro-Labs Diagnostics, Richmond Hill, Ontario, Canada). Each 2-ml tube contains approximately 50 plastic beads (diameter, 3 mm) with a hole through the center (this hole retains approximately 1 µl of suspension), which allows repeated recovery of an isolate before the preparation of a new stored culture is needed. This is in contrast to traditional long-term storage methods, in which the isolates are stored in multiple single-use vials, and has the added advantage of taking up less space.

Mycelium and conidia were harvested from 7-day-old cultures incubated at 27°C on Sabouraud dextrose agar (SDA; Oxoid Ltd., United Kingdom) by using a sterile scalpel and inoculated into a freezer bead tube containing a suspension medium prepared according to the manufacturer's instructions to give a density approximately equal to or greater than that of a McFarland no. 4 standard. The suspension was shaken vigorously to evenly distribute the fungus and was left to stand for 5 min. Excess fluid was removed with a Pasteur pipette. Before the tubes were frozen and stored at –70°C, a single bead was removed with sterile forceps and was placed on a fresh SDA plate, and the resulting drop of fungal suspension was spread by using a 10-µl loop to obtain single colonies and to check for viability and purity. These plates were incubated at 27°C for 7 days to assess the amount of inoculum present on a single bead. At various time intervals over 24 months, the tubes were removed from the freezer and a bead was removed from the frozen clump, plated, and incubated as described above. The tubes were immediately returned to the –70°C freezer before the contents had thawed. The number of colonies recovered, their growth rate, and the macroscopic and microscopic morphologies of the isolates were noted.

A detailed time course study was conducted with four isolates (*Trichophyton interdigitale* WHH1268, *Trichophyton mentagrophytes* WHH692, *T. rubrum* WHH3229, and *Epidermophyton floccosum* WHH1471). Single beads were removed at 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20, 24, 32, and 36 weeks and were cultured as described above. *Epidermophyton floccosum* was included, as it is known to die rapidly if it is kept at 4°C. In all cases, at all time intervals, successful reestablishment of the cultures ensued. At least 1,000 CFU was typically recovered from each bead. The growth rates and the hyphal densities were comparable to those of an initial control culture before it was frozen. Colonial and microscopic morphologies remained true to type throughout.

In addition to this time course study, a second trial was conducted with 58 stored isolates representing 15 species of dermatophytes. The isolates were selected to give a range of species but also to sample a range of isolates within some of these species (e.g., *Arthroderma benhamiae*, *Trichophyton interdigitale*, and *Trichophyton tonsurans*). For this trial, isolates stored for different time periods over the previous 24 months were recultured in triplicate to assess the uniformity of the distribution of viable organisms in frozen tubes. Three freezer beads were taken from each tube of preserved isolates and cultured as described above. In all cases, successful reestablishment from all three replicates occurred for all isolates tested. No adverse effects on morphology or growth rates compared to those of cultures not subjected to cryopreservation were noted.

Following these initial trials, this method of preservation was adopted for the storage of all stock strains in the laboratory. To date, all cultures kept in this manner have been successfully revived as required at times ranging from 1 week to 2 years, and these cultures represent 200 isolates of 21 species of dermatophytes (Table **1**). Espinel-Ingroff et al. (**1**) have discussed the advantages of using the Microbank freezer bead system in terms of its availability and ease of use. Their results suggested that dermatophytes would not be well preserved by use of this method. Our results for a wider range of isolates suggest that the limited numbers of dermatophytes that they tested were not representative or that a different preservation technique might have given better recovery rates. For example, half the specimens prepared by Epinel-Ingroff et al. (**1**) were preserved in liquid nitrogen and kept for up to 8 years, whereas all our specimens were kept at –70°C and tested within 2 years. The recovery of all the isolates used in our study was successful, with no apparent effect on culture phenotype. All four specimens of *T. rubrum* were recovered in our study, including the isolate used in the detailed time course study. This is in contrast to the 54% recovery rate of *T. rubrum* isolates in the earlier study (**1**). As a consequence, we can now recommend this method of preservation of dermatophytes for clinical laboratories worldwide.

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TABLE 1. Selected isolates recovered after preservation for 1 week to 24 months [a]

Name	Isolate code	No. of strains	Time of storage (mo)
Arthroderma benhamiae	NCPF410	1	24
A. benhamiae	NCPF456	1	3
A. benhamiae	NCPF460	1	0.25
A. benhamiae	Clinical isolates	13	2-10
Arthroderma simii	NCPF494	1	24
A. simii	NCPF471	1	1
Arthroderma vanbreuseghemii	NCPF452	1	24
A. vanbreuseghemii	NCPF750	1	24
A. vanbreuseghemii	NCPF749	1	24
Epidermophyton floccosum[b]	Clinical isolate WHH1471	1	24
E. floccosum	Clinical isolates	7	8-24
Microsporum audouinii	Clinical isolates	4	1
Microsporum canis	Clinical isolates	7	9-24
Microsporum gypseum-Microsporum fulvum	Clinical isolates	5	1-9
Microsporum persicolor	NCPF502	1	24
M. persicolor	Clinical isolates	4	1-9
Trichophyton ajelloi	NCPF364	1	12
Trichophyton equinum	NCPF526	1	24
T. equinum	NCPF565	1	24
T. equinum	NCPF673	1	18
T. equinum var. *autotrophicum*	NCPF488	1	24
T. equinum	Clinical isolate WHH2660	1	24
Trichophyton erinacei	NCPF652	1	24
T. erinacei	Clinical isolates	10	1-10
Trichophyton interdigitale	NCPF780	1	24
T. interdigitale[b]	Clinical isolate WHH692	1	24
T. interdigitale	Clinical isolates	55	1-24
Trichophyton mentagrophytes	NCPF224	1	24
T. mentagrophytes[b]	Clinical isolate WHH1268	1	24
T. mentagrophytes	Clinical isolates	27	1-24
Trichophyton quinckeanum	NCPF310	1	24
T. quinckeanum	NCPF341	1	24
T. quinckeanum	Clinical isolate	1	0.25
Trichophyton rubrum	NCPF113	1	24
T. rubrum[b]	Clinical isolate WHH3229	1	24
T. rubrum	Clinical isolates	2	1-24
Trichophyton schoenleinii	NCPF691	1	12
T. schoenleinii	Clinical isolate Bristol ST5	1	1
Trichophyton soudanense	NCPF800	1	12
T. soudanense	Clinical isolates	4	2-14
Trichophyton tonsurans	NCPF690	1	24
T. tonsurans	Clinical isolates	25	1-22
Trichophyton verrucosum	Clinical isolates	2	1-22
Trichophyton violaceum	Clinical isolates	5	7-22
Total		200	

[a] In each case, three replicate freezer beads were removed and the fungus was successfully revived from each one. NCPF, National Collection of Pathogenic Fungi, United Kingdom; Clinical isolates were isolated over the past 3 years in the William Harvey Hospital (WHH), Ashford, United Kingdom.

[b] Isolates used for detailed time course study.

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Pro-Lab Diagnostics Canada
20 Mural Street, Unit #4
Richmond Hill, ON L4B 1K3
Canada
Toll Free: 1-800-268-2341
Tel: (905) 731-0300
Fax: (905) 731-0206
support@pro-lab.com

Pro-Lab Diagnostics U.K.
7 Westwood Court
Neston, Cheshire
CH64 3UJ, United Kingdom
Tel: 0151 353 1613
Fax: 0151 353 1614
uksupport@pro-lab.com

Pro-Lab Diagnostics U.S.A.
21 Cypress Blvd., Suite 1070
Round Rock, TX, 78665-1034
U.S.A.
Toll Free: 1-800-522-7740
Tel: (512) 832-9145
Fax: 1-800-332-0450
ussupport@pro-lab.com

www.pro-lab.com