Public Health England

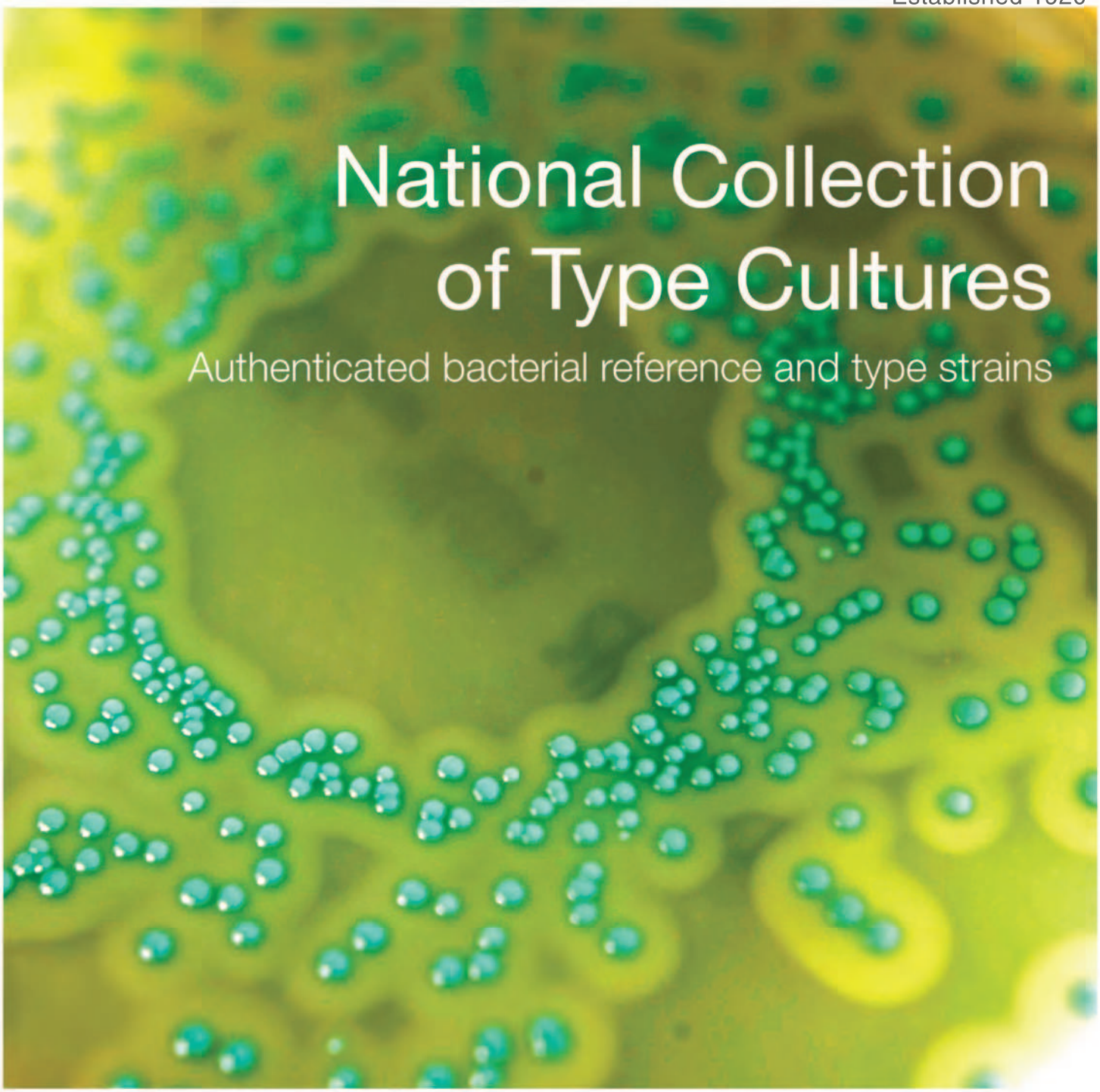
Protecting and improving the nation's health

NCTC
National Collection of Type Cultures
Operated by Public Health England

Established 1920

National Collection of Type Cultures

Authenticated bacterial reference and type strains



Distributed by:

TCS Biosciences Ltd
Botolph Claydon, Buckingham, MK18 2LR, United Kingdom
t: +44 (0) 1296 714222, f: +44 (0) 1296 714806, e: sales@tcsgroup.co.uk, i: www.tcsbiosciences.co.uk

The Culture Collections – a Public Health England biological resource centre

The Culture Collections is a Biological Resource Centre (BRC) operated by Public Health England (PHE) that provides many thousands of authentic microorganisms and cell lines for use in biomedical research and diagnostic testing. The biological resources and associated services provided by Culture Collections underpin the importance placed by Public Health England on meaningful scientific results in protecting and improving people's health.

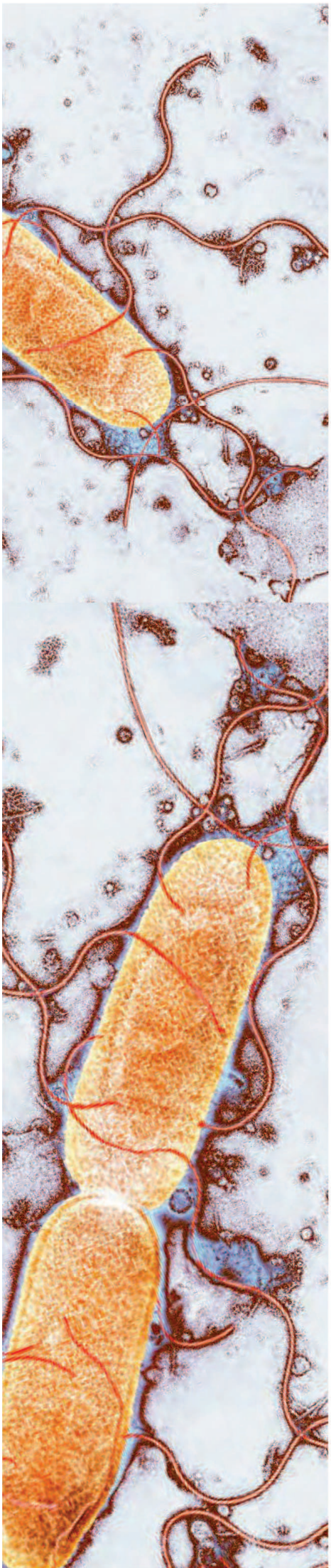
The four culture collections operated by Public Health England are:

- National Collection of Type Cultures (NCTC) – bacteria of medical importance
- National Collection of Pathogenic Viruses (NCPV) – clinically important viruses
- National Collection of Pathogenic Fungi (NCPF) – clinically significant fungi
- European Collection of Cell Cultures (ECACC) – human and animal cell lines

Together, these four collections form one of the world's largest BRCs, and it is uniquely placed in being able to draw on support from the wealth of expertise throughout our organisation.

We recognise that the true value of the collections lies not only in the microorganisms and cell lines but also in the information about those biological resources, such as when and where they were collected, their unique features and potential uses. Scientists worldwide use the PHE biological resources and their associated services. Our role is to protect and develop the collections by interacting with users so that they benefit from the biological materials and we benefit from their expertise. Specialist teams study and maintain the collections and ensure they are readily accessible to the scientists who need them.

All the PHE collections are developed, managed and maintained by highly trained, dedicated staff working in accordance with internationally recognised quality standards including certification to ISO 9001:2008 and accreditation to ISO 17025:2005.



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Introduction to the National Collection of Type Cultures (NCTC)

NCTC is one of the longest established collections of microorganisms in the world. Set up in 1920 to "provide a trustworthy source of authentic bacteria for use in scientific studies", the remit for NCTC remains essentially unchanged today. Although initially a general microbial collection, the focus changed in 1947 to allow the NCTC curators to concentrate on bacteria of medical and veterinary interest. This strategy was implemented to ensure that the collection would be preserved by specialists in the field; non-medical cultures were transferred to other institutes with different expertise.

NCTC cultures reflect the history of clinical bacterial infections from the end of the 19th century to the present. There are currently more than 5000 type and reference strains available, representing over 1000 different bacterial species, and those numbers constantly increase. We add new strains to the collection continuously to ensure we are delivering the vital biological resources necessary for researchers gathering new data on microbial biodiversity, studying the similarities and differences between historical and modern strains and advancing global knowledge about the epidemiology, virulence, prevention and treatment of infectious diseases. In addition to bacterial strains for research studies, NCTC provides new and established control strains for use in clinical diagnostic testing, microbiological assessments of food, water and environmental samples, and the quality control of culture media.

We provide information about available NCTC cultures in our online catalogue at www.phe-culturecollections.org.uk/nctc. In addition to the cultures accessible via the website, NCTC has hundreds of other strains in private and historical collections that have been donated by individual scientists, academic institutions and the Wellcome Trust. NCTC bacterial strains are preserved as characterised, authenticated freeze-dried (lyophilised) cultures in fused-glass ampoules and stored in controlled secure conditions. Duplicate collections are maintained in two separate geographical locations wherever possible to ensure this resource is available for future generations.

NCTC also serves as a United Nations Educational, Scientific and Cultural Organization (UNESCO) Microbial Resource Centre (MIRCEN). In 1982 NCTC was recognised as an International Depository Authority (IDA) under the Budapest Treaty which contains the regulations on the international recognition of the deposit of microorganisms for patent procedure. Intellectual property offices require the written disclosure for the invention of a new microorganism to be supplemented by the deposit of that microorganism in a recognised culture collection such as NCTC.

NCTC cultures and services are used by scientists all over the world. There are established national and international biosecurity and transport regulations for the provision of microorganisms that we must adhere to. This ensures that people who receive NCTC strains do so safely and understand the terms and conditions of use.


Antibiotic Resistance Research
Drug Discovery
Clinical Diagnostic Testing
Water Testing
Environmental Testing
Food Testing
Microbial Taxonomy
Technology Advances in Proteomics and Genomics
Infectious Disease Research
NCTC products and services support improvements in public health outcomes

NCTC preservation formats

Most NCTC cultures are preserved and distributed as freeze-dried (lyophilised) cultures in glass ampoules flame-sealed under vacuum. NCTC introduced freeze-drying in the 1930s and the process, essentially unchanged, is still considered to be the most reliable method for the archiving and long-term storage of bacteria. Freeze-dried cultures are relatively simple to store and dispatch worldwide. Many strains remain viable for more than 20 years, provided the initial cultures contain between 10^6 and 10^{10} colony forming units per ml. However, the survival time can vary for different species within a genus and between different strains of the same species. The NCTC laboratory undertakes annual viability checks on all batches to ensure the bacteria remain viable and will produce new batches if the levels begin to fall below a stipulated threshold.

NCTC provides a selected range of strains as LENTICULE discs, a single-use format that was developed to provide control strains for food and water microbiology testing laboratories.

Some commercial companies also provide products that contain NCTC strains which are manufactured under licence from NCTC.

- freeze-dried ampoules

Most NCTC bacterial strains are presented as a freeze-dried pure culture in a glass ampoule that has been flame-sealed under vacuum. Ampoules contain approximately 0.15g of the freeze-dried bacterial suspension. Every ampoule is individually packed in a plastic tube, dispatched at ambient temperature and requires storage at +4°C.

- LENTICULE discs

LENTICULE discs contain a stipulated level of viable biologically active bacteria that have been control-dried in a water-soluble matrix. Each disc is packed individually in a plastic tube over a desiccant, dispatched at ambient temperature and requires storage at -20±5°C. The product quality is not affected significantly if it is held for short periods at ambient temperature such as during normal dispatch.

- incorporated into other products (TCS Selectrol®, bioMérieux BioBall®, Microbiologics KWIK-STIK™, proficiency testing schemes)

Quality control and authenticity of NCTC strains

Although scientists tend to be careful when selecting instruments, kits and reagents for their research or test procedures, they often overlook the importance of sourcing and managing their control strains and biological materials. Some estimates indicate that up to 70% of strains used in published research are not from recognised culture collections, which means that tens of thousands of scientists may be using strains for research without proper authentication and provenance. There is also published evidence[1] that diagnostic testing laboratories do not always manage their control strains effectively. This is possibly because there is a perception that authenticated biological materials are expensive or difficult to handle and store. There are serious implications associated with reporting misleading results and reaching unfounded conclusions that can arise from the use of incorrect or unauthenticated strains.

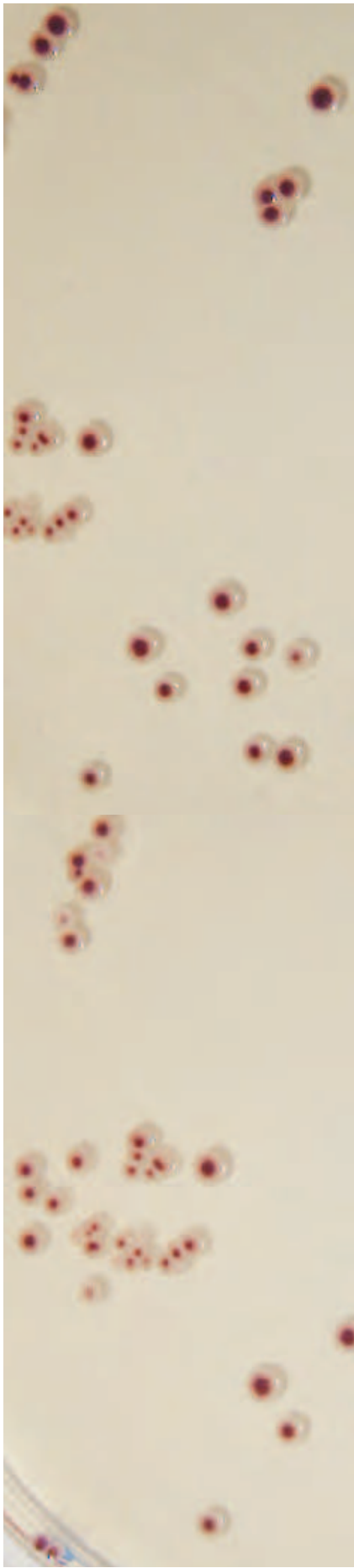
All cultures received in our NCTC laboratory for inclusion in the collection are prepared as freeze-dried stocks which are generated in such a way as to ensure that the number of subcultures (passages) is kept to a minimum. This reduces the risk of genetic mutations occurring. The depositor is involved in the initial authentication process to ensure that their culture has retained the expected characteristics after preservation. The NCTC team undertakes quality control tests for the initial and every subsequent batch, which include:

1. Morphology and behavioural tests (physiology, nutritional requirements, enzyme tests)
2. Protein tests (serology, mass spectrometry (MALDI-TOF MS[2]))
3. Genomic tests (16S ribosomal RNA gene sequencing, whole genome sequencing)

NCTC produces certificates of analysis for every batch that provide information about the results of the quality control tests undertaken so scientists can be confident about the authenticity of their strains.

[1] Cross, LJ, Russell, JE and Desai, M. Examining the genetic variation of reference microbial cultures used within food and environmental laboratories using fluorescent amplified fragment length polymorphism analysis. FEMS Microbiol Lett. 2011, 1-7.

[2] Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

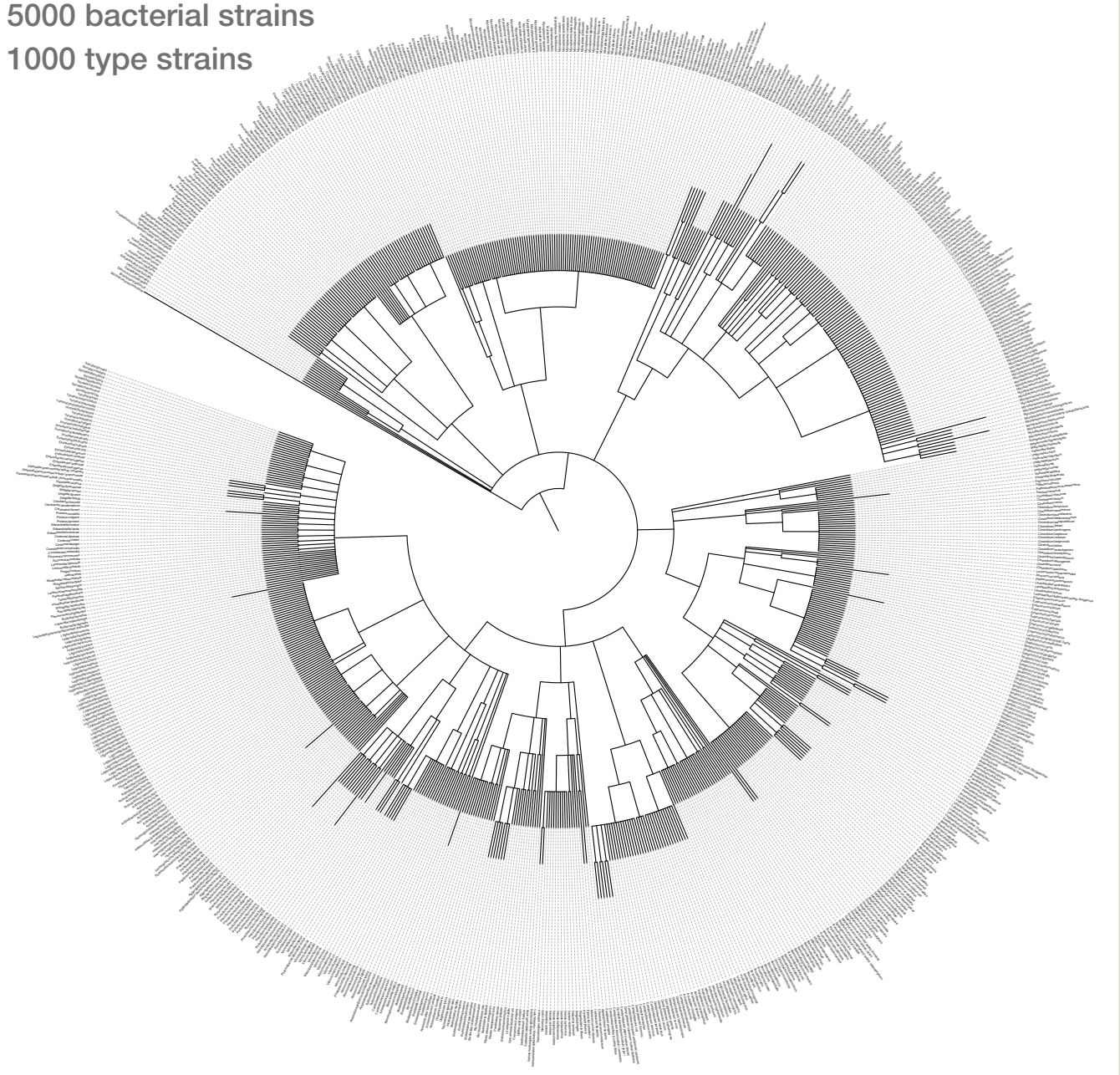


Range of strains available

With more than 5000 bacterial cultures including 1000 type strains to choose from it might be difficult to decide exactly what you need. The online catalogue is your first source of information – go to www.phe-culturecollections.org.uk/bacteria-search.

NCTC microbiologists are on hand to provide advice and can call on the wider expertise of scientists throughout PHE if they need help answering your questions.

The phylogentic tree diagram below represents NCTC bacterial species available and was generated using the interactive tree of life web tool (Letunic and Bork. Interactive Tree of Life v2: online annotation and display of phylogenetic trees made easy. Nucleic Acids Res. 2011. 39 (suppl 2): W475-W478).



USEFUL FACTS

Type strains:

Not all NCTC strains are type strains. The type strain is the strain on which the description of a species is based and is not always the most typical representative of a species. Type strains serve as fixed reference points for the assignment of bacterial names. They are cultured from the original isolates used in species and subspecies descriptions, as defined by the Bacteriological Code of Nomenclature of Bacteria. It is mandatory for type strains to be deposited in recognised culture collections such as NCTC.

NCTC type strains in evolutionary studies:

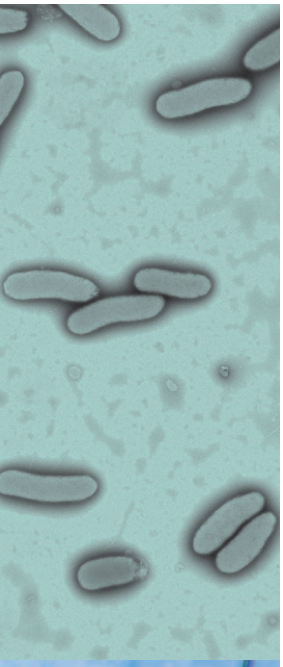
Phylogenetic trees are the graphical representations of evolutionary relationships of species and reflect their closeness. Traditionally, phylogenetic trees were constructed on the basis of physical similarities and differences between microorganisms and type cultures played an important role in their construction. Advancing technology means that whole genomic sequences can now be used to build phylogenetic trees and the role of type cultures continues to be of crucial importance. Whole genome sequences are available for many NCTC cultures.

Reference strains:

All NCTC strains, regardless of whether they are type strains, are acknowledged reference strains. Reference strains are defined as any microorganisms acquired from a recognised culture collection. Many NCTC reference strains are cited as controls for internationally recognised test procedures.

Changes to names of organisms:

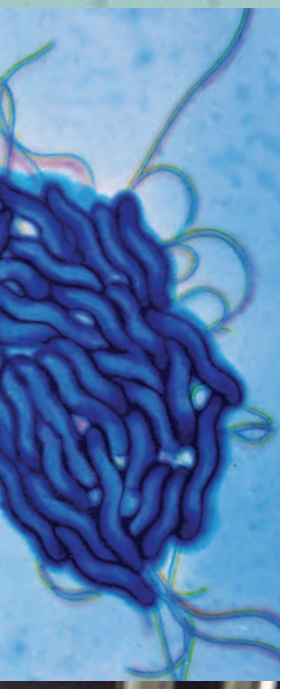
Bacterial strains may be re-classified when new information about them is discovered or newer techniques are applied to studies of their systematics. These re-classifications are often accompanied by changes in nomenclature, for example, when new genera and/or species are recognised or when existing species are transferred to different (and sometimes new) genera. It is important to appreciate that these changes are proposals so the same organism can be known by several different names, provided that those names were proposed in accordance with the Rules of Nomenclature (International Code of Nomenclature of Bacteria). All such names proposed in accordance with the Rules can be considered to be ‘correct’. There is a common misconception that the latest proposed name or new combination is the ‘correct’ name. However, it is for the scientific community to decide usage, depending on their acceptance or not of the proposal. Not all new names or combinations gain wide acceptance, though most do.



Escherichia coli

NCTC holds 360 strains of *E. coli* isolated over the past 100 years from a wide range of sources. *E. coli* is a diverse group of bacterial strains found in the intestines of people and animals, the environment, food and water. Although most strains of *E. coli* are harmless, a significant number can cause illnesses including diarrhoea, urinary tract and kidney infections, respiratory illnesses including pneumonia, and septicemia.

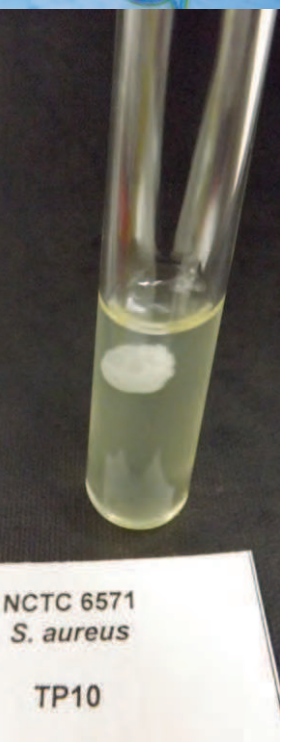
- the type strain of *E. coli* is NCTC 9001, isolated in 1895 by Fritz Kauffman, who later co-developed the Kauffman and White scheme for classifying salmonellae by serotype
- NCTC 9001 is used as a control for food and water tests, quality control of culture media, detection of aerosols and as a control for commercially available identification kits
- the whole genome sequence for NCTC 9001 is available from www.ebi.ac.uk/ena/data/view/ERS451419



Campylobacter

More than 72,000 cases of people suffering from illnesses caused by campylobacter in the UK were confirmed by laboratory reports in 2014. Most people survived, but not all. Two species of campylobacter, *C. jejuni* and *C. coli*, are responsible for most infections. A better understanding of campylobacter will help to reduce the burden of disease.

- NCTC holds 148 strains of campylobacter
- there are 57 NCTC strains of *C. jejuni* and 11 strains of *C. coli*
- the type strain for *C. jejuni* is NCTC 11351
- the whole genome sequence for NCTC 11351 is available from www.ebi.ac.uk/ena/data/view/SAMEA2479571



The Oxford Staphylococcus – NCTC 6571

Studies to characterise and assess the potential therapeutic use of penicillin began in the early 1940s and much of the early work was undertaken in Oxford University in the UK. The ‘Oxford Staphylococcus’ was deposited into NCTC in 1944 by N. G. Heatley who recommended this particular strain as a control to standardise methods for biological assays of penicillin. The Oxford Staphylococcus, NCTC 6571, continues to be used widely as a control in clinical diagnostic microbiology laboratories throughout the UK for tests such as the detection of coagulase and DNAase. It also serves as a reference strain for antimicrobial susceptibility testing.

- NCTC holds 250 strains of staphylococci from 27 different species
- there are 172 NCTC strains of *Staphylococcus aureus*
- NCTC provides multidrug resistant strains of *Staphylococcus aureus*

Antimicrobial susceptibility testing with NCTC control strains

NCTC, working in collaboration with Public Health England's Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL), provides the antimicrobial susceptibility testing control strains recommended by:

- the European Committee on Antimicrobial Susceptibility Testing (EUCAST)
- British Society of Antimicrobial Chemotherapy (BSAC)
- United States Clinical and Laboratory Standards Institute (CLSI)
- United Kingdom Standards for Microbiology Investigations (SMIs))

Multidrug resistant strains from NCTC

NCTC offers a large selection of clinically relevant multidrug resistant (MDR) reference strains isolated from a range of hospital and community-acquired sources. These cultures are useful for studying the genetic variations between strains, the *in vitro* evaluation of disinfectants and novel antimicrobials, and establishing the performance characteristics of molecular-based assays. NCTC provides strains with the following characteristics:

- penicillinase without extended spectrum β-lactamase activity
- extended spectrum β-lactamases
- TEM, SHV, CTX-M, VEB and AmpC β-lactamases
- class A, B and D carbapenemases
- plasmid-mediated fluoroquinolone resistance
- vancomycin resistance (enterococci)
- multidrug resistance plasmids (*E. coli*)
- methicillin resistance (*S. aureus* – MRSA)

NCTC *Clostridium difficile* control strains

C. difficile infections are an important global healthcare problem and remain the most common cause of infectious diarrhoea in hospitalised patients in the UK. PCR-ribotyping of *C. difficile* can be useful for evaluating transmission so the PCR-ribotypes and toxin status are provided for all NCTC *C. difficile* reference strains.

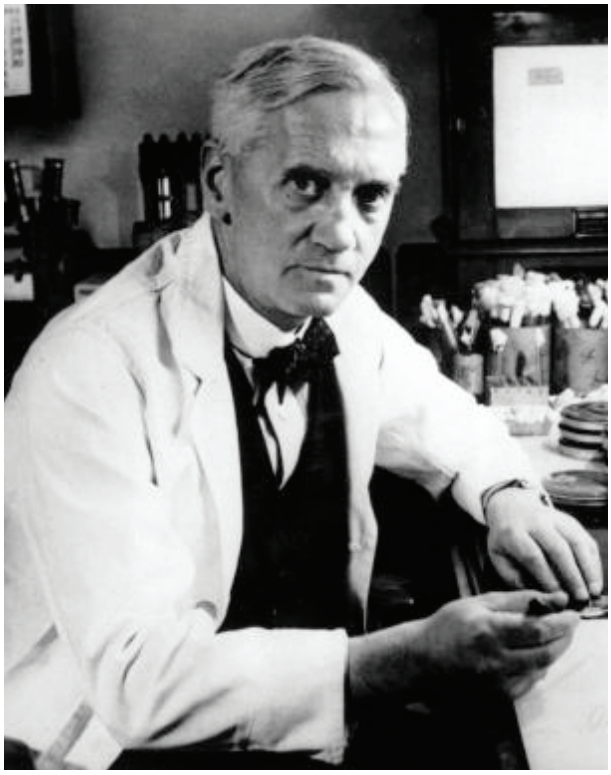
Depositing strains with NCTC

Frederick William Andrewes – deposited the very first cultures in NCTC in 1920 including NCTC 1, *Shigella flexneri* – see page 18.

Samuel Tertuis Cowan – Curator of NCTC between 1947 and 1965 – co-author of Cowan and Steel's Manual for the Identification of Medical Microbiology.

Howard W. Florey – worked with Ernst Chain to develop penicillin so it could be produced as a therapeutic agent and deposited three strains of bacillus into NCTC.

Betty Constance Hobbs – pioneering microbiologist and internationally recognised authority on food poisoning and food hygiene deposited more than 20 strains of bacteria associated with food-borne illness.



Alexander Fleming – best known for his discovery of penicillin, deposited 16 cultures into NCTC between 1928 and 1948.

How to become a 21st century NCTC depositor

NCTC is a dynamic collection that must remain relevant to the scientific community, providing a trustworthy source of authentic bacteria of medical and veterinary interest. The collection was established by forward-thinking scientists who realised that some of the strains they isolated may be of value in future fights against infection. Today, scientists are still encouraged to deposit strains with NCTC that are novel or of clinical significance. We are able to accept strains that are in ACDP[3] Hazard Groups 2 and 3. There are many reasons why scientists and clinicians deposit strains and the process for doing this is relatively simple. Of particular importance are:

- novel species of bacteria – these must be deposited with two culture collections in different countries as part of the process of naming and describing the organism to comply with the International Code of Nomenclature of Bacteria
- bacterial strains that have caused outbreaks of infection, particularly those with novel virulence factors
- emerging strains of medical and veterinary interest, particularly those with unusual characteristics
- strains that may be of value as controls in clinical diagnostic, food, water, environmental or veterinary microbiology settings
- key strains referred to in peer-review publications – to allow other scientists ready access to an authenticated isolate of the strain in order to replicate or develop the study

Contact NCTC for advice about depositing strains or download the deposit form on the website at:
www.phe-culturecollections.org.uk/deposit-strains-with-nctc

The deposit form needs to be submitted before any strains are sent to NCTC so the isolates can be assessed by our scientists. A member of our team will contact you to advise you whether your strains can be accepted and explain the next steps of the process.

[3] ACDP Advisory Committee of Dangerous Pathogens which prepares an approved list of biological agents and associated guidance at the request of the UK Health and Safety Executive.

Middle right image: Professor Neil Woodford, Head of Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI), Public Health England

Benefits of depositing strains with NCTC

- contribute to an established internationally acclaimed resource that will help investigators fight infectious diseases in the future
- raise the profile of your peer-reviewed publications by sending them to NCTC with your deposited strains so we can highlight your work through our website
- access isolates of your deposited strains quickly whenever you need them
- minimise the need for you to dispatch your important cultures to other laboratories, use our expertise of biosecurity and licenses required
- specify when the organism is made available through flexible release dates. For example you can request an organism is made available only after you have published your paper

Contact NCTC for advice about depositing strains or download the deposit form on the website at: www.phe-culturecollections.org.uk/deposit-strains-with-nctc

NCTC reference materials for food and water testing

NCTC works with collaborators and international distributors to provide reference material (RM) products designed especially for use as controls in food and water testing laboratories. The RMs, offered in LENTICULE disc format (see page 5), are prepared from a single subculture of the relevant NCTC strain. They are easy to use and save time so can be very cost effective, particularly in busy laboratories. The RMs provide consistent data, thereby increasing confidence that test results are reliable.

The RM products are useful for:

- providing controls for the detection of pathogens that may be present in low levels, such as *Salmonella* spp. and *E. coli* O157
- deliberate inoculation ('spiking') of food and water samples to demonstrate staff competence in testing the specific food/water matrices tested in your laboratory
- enumeration controls for food and water testing
- quality control materials to demonstrate performance of culture media
- staff training – many laboratories report mainly negative results so staff may not become familiar with the detection of pathogenic organisms – the RMs can be used for this purpose

Factors to consider when selecting reference materials:

Public Health England microbiologists are available to advise about the selection and use of the NCTC RMs. Some factors to consider are:

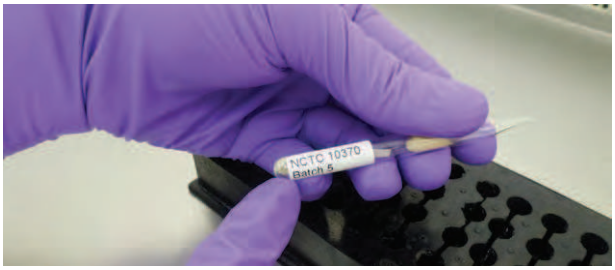
- is the control strain correct for a process stipulated in an international method for food or water testing? NCTC RMs are designed to meet this requirement
- are you sure it is the correct control strain for the purpose? The Culture Collections website provides extensive information about the strains; we also have experts on hand to advise if you are unsure
- is the control strain being used at an appropriate detection level? If you are choosing a control for a detection method, check that it will challenge the limit of detection of your test

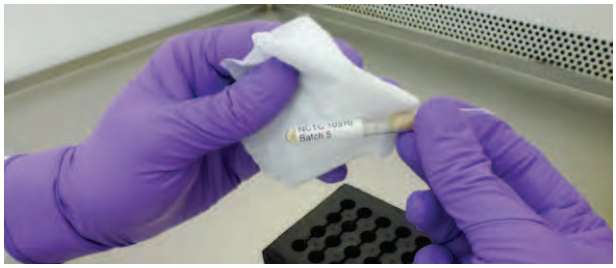
How to open and grow your freeze-dried NCTC strains

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

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

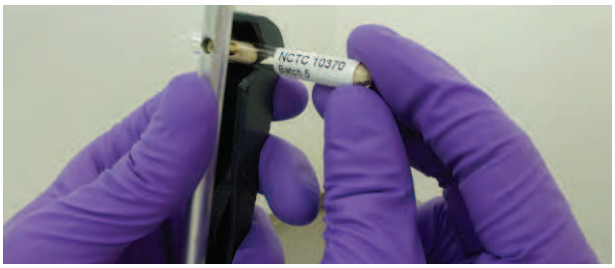
1. Prepare the appropriate medium necessary for reviving the strain and check the required incubation conditions.

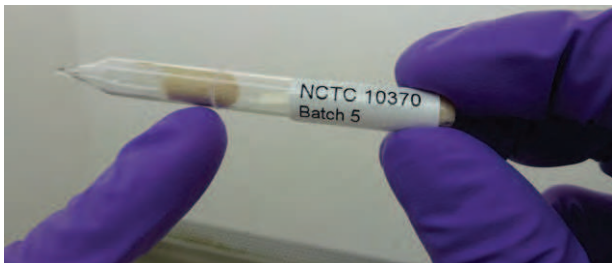




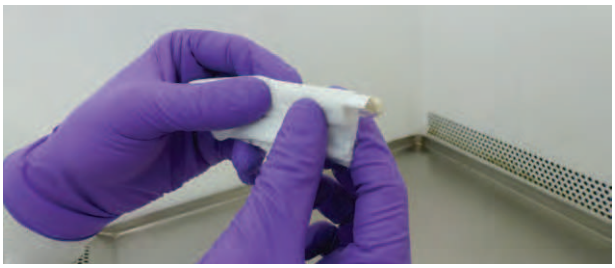
2. Identify the culture by the NCTC number on the paper inside or label on the ampoule.

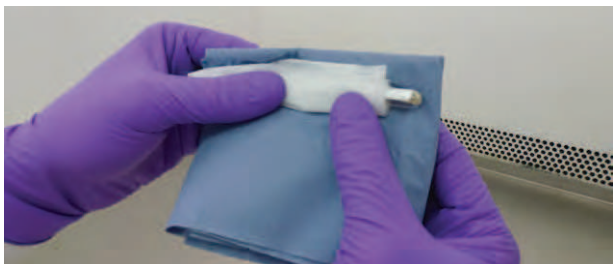
3. Clean the outside of the ampoule using a disinfectant wipe or tissue soaked in 70% alcohol.





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

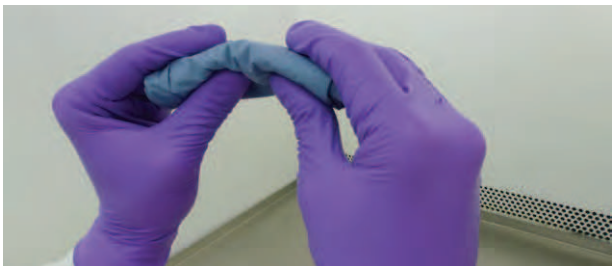




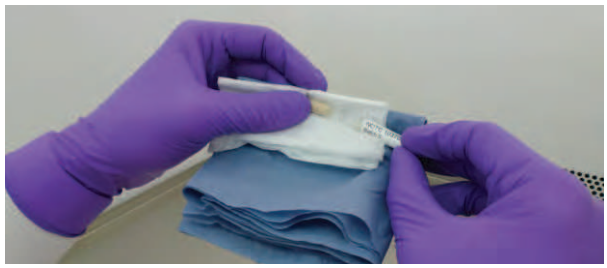
5. Wrap the ampoule in a disinfectant wipe or a tissue soaked in 70% alcohol.

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

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

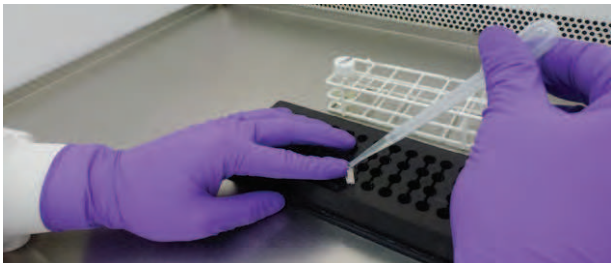


7. Snap the ampoule while wrapped where the score mark was made.

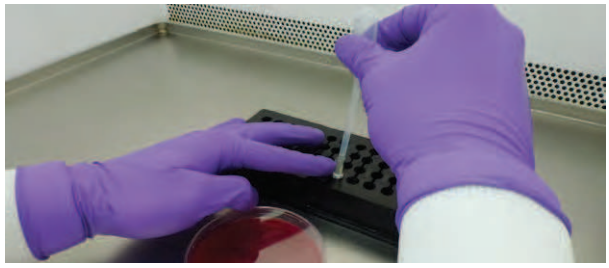


8. Carefully unwrap the ampoule as fragments of glass may be present in the tissue. Discard the tissue and the ampoule tip into a sharps bin.

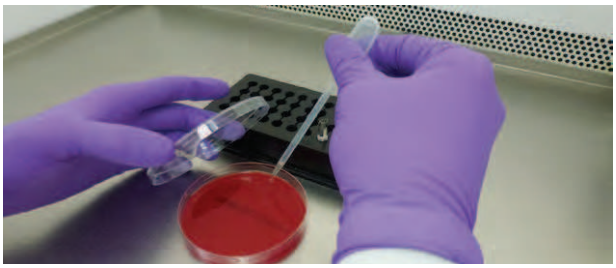
9. Once opened, air will enter the ampoule because the vacuum is no longer intact. This air will be filtered by the cotton wool plug which may have been in contact with dried bacterial culture so must be discarded safely. Remove the plug with forceps if the plug does not come away with the tip of the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



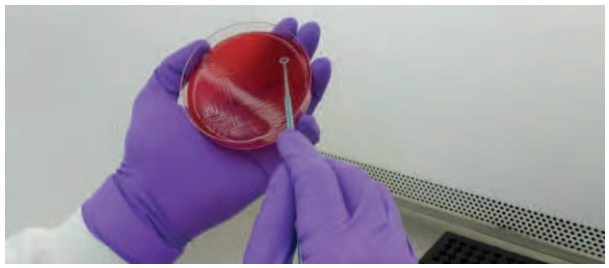
10. To reconstitute, transfer approximately 0.5ml of broth, when necessary enriched with blood, to the ampoule.



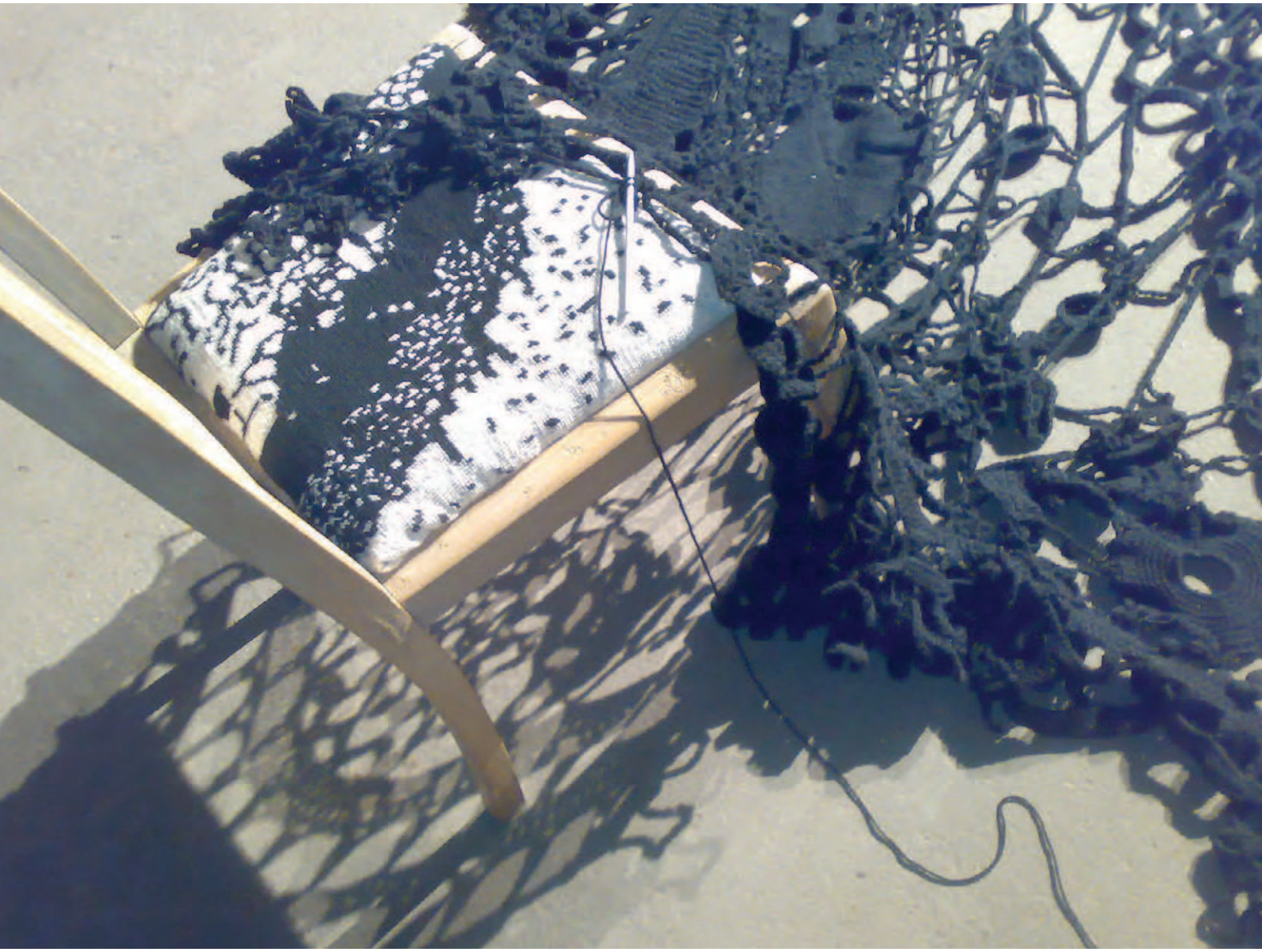
11. Allow the microorganisms to rehydrate for 5-10 minutes. Mix very carefully to avoid creating aerosols or causing the contents to froth. Check all material is dissolved.



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



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



“Bed and Chair Flora” by Anna Dumitriu www.normalflora.co.uk

The NCTC 3000 project – reference genomes

NCTC and the Wellcome Trust Sanger Institute (WTSI) are delivering a five-year project funded by a Wellcome Trust Biological Resource Grant. The project began in 2014 and will produce a unique electronic resource that will bring together the complete strain information for NCTC type and reference strains. We will be providing integrated web links that will direct users to strain specific information held at external websites including genome sequences and journal papers in PubMed. As part of the project we are sequencing and assembling the genomes of 3000 NCTC strains using long read (Pacific Bioscience) technology.

Be part of the NCTC 3000 project

It is particularly important that we engage with the scientific community to ensure that this vital resource meets the users' needs. You can follow us on Twitter **@NCTC_3000** for the latest developments and to see which new sequences we have released. You can also share your feedback via Twitter and help us to shape this project. We will also use more traditional methods of engagement and you can find information by going to: www.phe-culturecollections.org.uk/nctc3000project

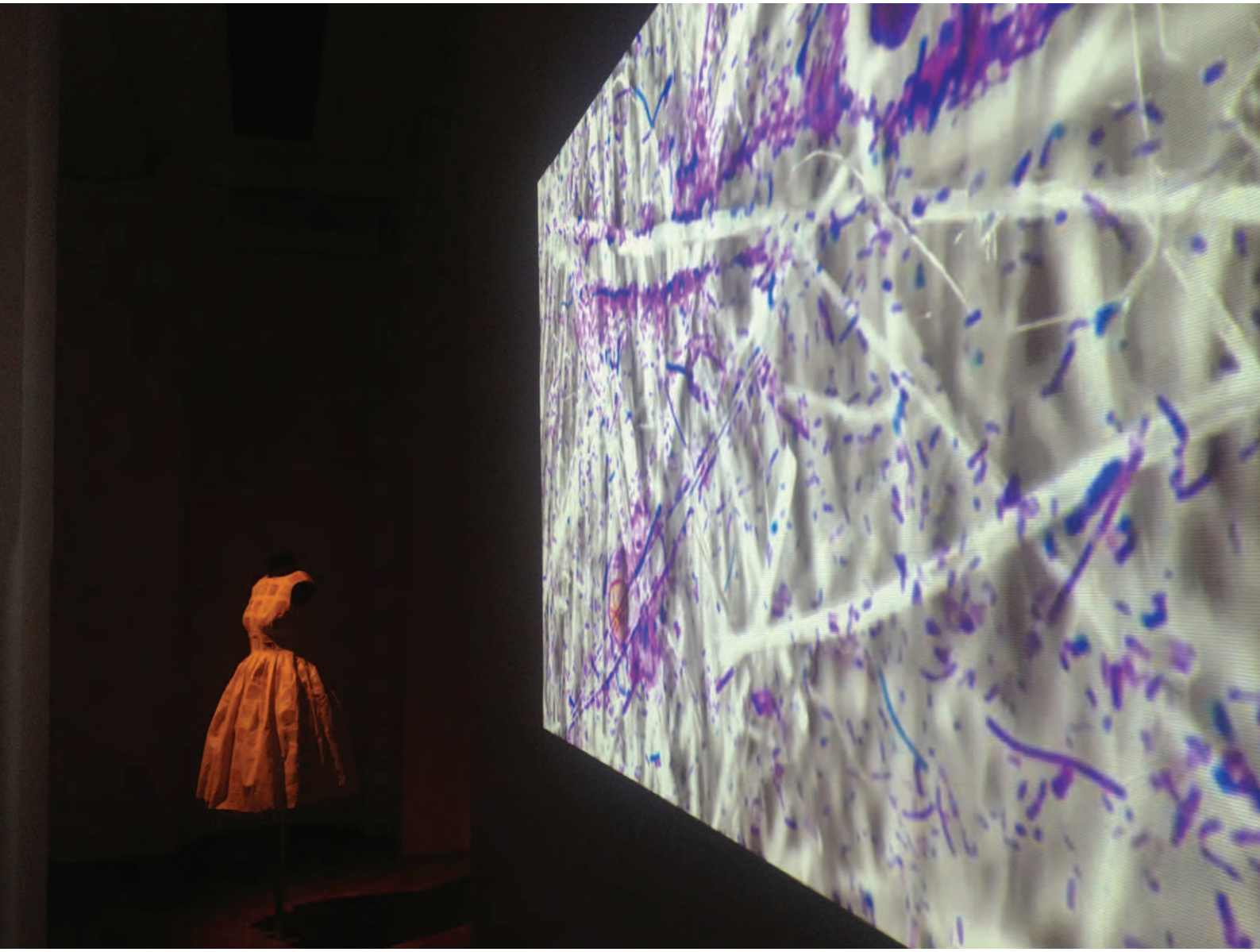
The value of historical NCTC strains

The genome of NCTC 1, the first strain to be deposited in NCTC, was assembled by scientists at the WTSI as part of the NCTC 3000 project. NCTC 1 is a strain of *Shigella flexneri* that was isolated in 1915 from a soldier in the trenches of the Western Front in World War 1. *S. flexneri* infection causes bacterial dysentery, a life-threatening disease that kills hundreds of thousands of children under five each year in developing nations and is becoming increasingly hard to treat. NCTC 1 is resistant to penicillin and erythromycin even though it was isolated before the discovery and widespread application of antibiotics to treat infectious diseases.

The NCTC 1 genome was compared with those of three other *S. flexneri* strains isolated in 1954, 1984 and 2002. Dr Kate Baker (Wellcome Trust Sanger Institute) noted that when the genomes of the four isolates were compared side by side, an evolutionary pattern could be seen. Genomic analysis revealed that NCTC 1 belongs to a specific (2a) lineage of *S. flexneri,* with which it shares common characteristics and a large core genome. Genomic islands gained in the *S. flexneri* 2a lineage over time are predominately associated with additional antimicrobial resistances, virulence and serotype conversion. This study was published in The Lancet and attracted significant media publicity in November 2014.

Kate S Baker, Alison E Mather, Hannah McGregor, Paul Coupland, Gemma C Langridge, Martin Day, Ana Deheer-Graham, Julian Parkhill, Julie E Russell, Nicholas R Thomson. **The extant World War 1 dysentery bacillus NCTC1: a genomic analysis.** The Lancet. 2014; Volume 384, Issue 9955, Pages 1691 – 1697.

You can view a short documentary film about the isolation and sequencing of *S. flexneri* NCTC 1 at: www.phe-culturecollections.org.uk/news-nctc1



"The VRSA Dress" and "The Art and Science of Linen" by Anna Dumitriu
www.normalflora.co.uk, installation view at Kapelica Gallery, Ljubljana

NCTC partnerships and collaborations

Chlamydia Biobank

NCTC is an advisory body for the Chlamydia Biobank project which is the first dedicated collection of *Chlamydia trachomatis* isolates, selected to represent the full diversity of the species. The Biobank is hosted at the University of Southampton, in collaboration with the Wellcome Trust Sanger Institute, and is funded by a Wellcome Trust Bioresource Research Grant. For further information go to www.chlamydiabiobank.co.uk.

World Federation for Culture Collections (WFCC)

The WFCC was established with UNESCO support in the 1970s with the aims of promoting and supporting the establishment of culture collections and related services and setting up an information network between the collections and their users. NCTC is registered with the WFCC which pioneered the development of an international database on culture resources worldwide, the World Data Centre for Microorganisms (WDCM).

The WDCM Reference Strains Catalogue has been produced to enable broader and easier access to the reference strains listed by the ISO TC 34 SC 9 Joint Working Group 5 and by the Working Party on Culture Media of the International Committee on Food Microbiology and Hygiene (ICFMH-WPCM) in their publication Handbook of Culture Media for Food and Water Microbiology. It was established to fulfil a need for a simple unique system of identifiers for strains recommended for use in quality assurance. This means that users can refer to the WDCM online catalogue to see which control strains they require and will be provided with a list of different culture collection organism numbers so they can access the required strains from their preferred source. There are nearly 100 NCTC strains listed on the WDCM Reference strains catalogue, mostly associated with food and water microbiology testing. For further information go to www.wdcm.org.

Microbial Resource Research Infrastructure (MIRRI)

NCTC is a Collaborating Partner for an important European initiative known as the Microbial Resource Research Infrastructure (MIRRI). MIRRI aims to provide researchers with easier and more efficient access to the best microbial resources, services and data available throughout Europe. For further information go to: www.mirri.org.

European Culture Collections' Organisation (ECCO)

NCTC is a member of the European Culture Collections' Organisation (ECCO) established in 1981. The aim of the organisation is to promote collaboration and exchange of ideas and information about all aspects of culture collection activity. ECCO meetings are a valuable forum for discussion and innovation on the future development of member collection activities. For further information go to: www.eccosite.org.

Other NCTC services

In addition to preserving, authenticating and supplying bacterial strains, NCTC provides a number of related services to the scientific community.

Freeze-drying (lyophilisation) service

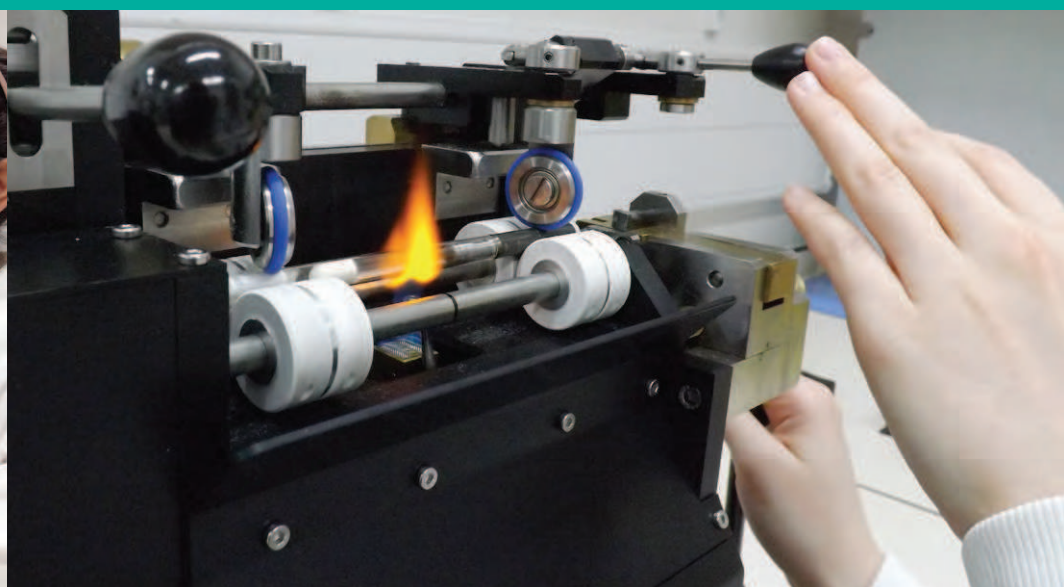
Specialised equipment and expertise are required for freeze-drying bacterial strains successfully so NCTC offers a freeze-drying service to scientists who want to preserve their own bacterial or fungal cultures. In addition to providing cultures in the standard flame-sealed evacuated glass ampoules, NCTC can also provide freeze-dried cultures in evacuated capped vials. Although these vials are considered to be easier to handle, the cultures are likely to be viable for a shorter period of time, depending on the strain. NCTC microbiologists will be able to advise you about this service.

International patent depository

NCTC is an International Depository Authority (IDA) patent depository for bacterial strains and plasmids recognised under the Budapest Treaty. This is an international treaty to which 75 countries are signatories and under this treaty certain formally nominated culture collections, such as NCTC, are designated as IDAs. This is because it is essential for patented bacterial strains to be deposited in a culture collection for future testing and to allow examination by other parties who are entitled to do so. NCTC handles bacterial strains deposited for patent purpose and their corresponding metadata in a confidential manner using a system that is independent from the main NCTC collection.

Special, unique and private collections

NCTC provides safe custody for private collections of bacterial cultures on behalf of third parties; this service is available for single cultures or entire collections.



NCTC at the forefront of technology

NCTC has always fostered a culture of scientific research and development, particularly relating to emerging methods for the identification of bacteria. Contributions by former Curator, Samuel T. Cowan, in 1947 lead to the First International Code for Nomenclature and later the publication of Cowan and Steel's Manual for Identification of Medical Bacteria (1965). NCTC was awarded a research grant by the UK's Department of Health and Social Security in 1965 to develop computer-based identification. The system used the Atlas Computer and was developed in partnership with academia and private industry. This was considered at the time to be one of the world's most powerful computers and the results are still available in the NCTC archive.

More recently, NCTC has explored genomic and proteomic technology working with colleagues across PHE and beyond. The NCTC 3000 project team is using long read genomic technology to make the whole genome sequences of NCTC's type and reference strains freely available for use as high quality reference genomes.

PHE researchers have worked with academia and industry over the past 12 years to develop proteomic technology for pathogen identification. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is now used in many diagnostic and hospital testing laboratories for identifying pathogenic bacteria and we use it for the authentication of NCTC cultures. The primary reason for the widespread adoption of this technology is that it compares favourably with the gold standard for identification, 16S rRNA analysis. However, NCTC include both 16S rRNA analysis and MALDI-TOF MS in the authentication procedure.

NCTC staff have been at the forefront of bacterial classification, taxonomy and nomenclature since Samuel T. Cowan authored 'A Dictionary of Microbial Taxonomic Usage' in 1968. This was a reference book for taxonomists that provided scientific information for editors of scientific journals and also for authors contributing to journals. Taxonomy and nomenclature present an on-going challenge as organism names change over time, particularly with the development of new technologies. International collaborations are essential as bacterial taxonomy experts are becoming increasingly rare.

NCTC – charging for cultures

NCTC is unusual in that the collection is not subsidised significantly by the government or any other external bodies so must recover all the operating costs by charging for the cultures and services. Although nominal fees for the cultures were instituted in 1947, a more robust pricing structure was introduced in 1970.

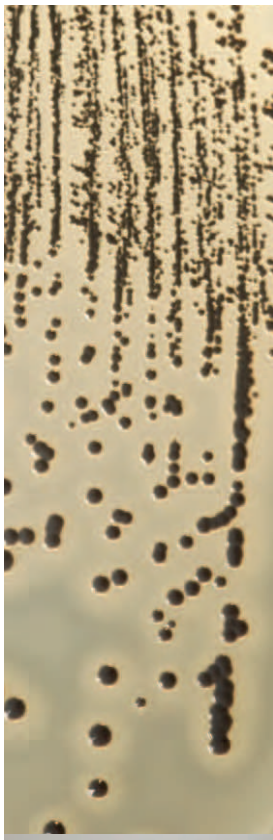
NCTC exists to "provide a trustworthy source of authentic bacteria for use in scientific studies" and our aim is to make the cultures as accessible as possible. We recognise that scientists will be deterred from using NCTC cultures if charges for cultures and services are too high.

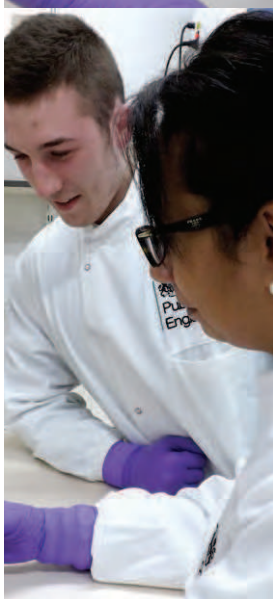
PHE is a not-for-profit organisation. Our prices reflect essential operating components such as:

- staff costs, essential to maintain and develop NCTC
- biosecurity and safety compliance requirements
- authentication and quality control tests
- preservation and storage of the cultures
- logistics, necessary to transport the cultures globally and ensure they are delivered safely and efficiently
- communication channels to support the users and general scientific community – including the maintenance and improvement of the online catalogue

NCTC cultures are provided free-of-charge to scientists who deposit their strains with the collection and fees can be reduced or even waived in some situations.

NCTC scientists can provide advice on how to maintain stock cultures within user laboratories to help to reduce the cost of maintaining authentic cultures.





Technical support and customer service

NCTC staff are happy to provide accurate advice and intelligent information about the materials or services that you require. We have a technical helpline for all culture collection users that can connect you with the person best-qualified to respond to each individual query.

Tel: +44 (0)1980 612684

Email: culturecollections.technical@phe.gov.uk

Biosecurity and biosafety

The pathogenic nature of some of the NCTC bacterial strains requires us to have strict controls in place to ensure the cultures are distributed safely and securely. Our staff have expertise in biosecurity and safety compliance requirements and can offer advice.

Licensing NCTC Cultures

We welcome the use of NCTC cultures in commercial products that are designed to improve the quality of microbiology testing, particularly in the clinical diagnostic, food, water and environmental sectors. We make NCTC cultures available, under licence, for a range of products such as those that provide process controls and also for inclusion in proficiency testing (external quality assessment) samples.

Making your own stock cultures

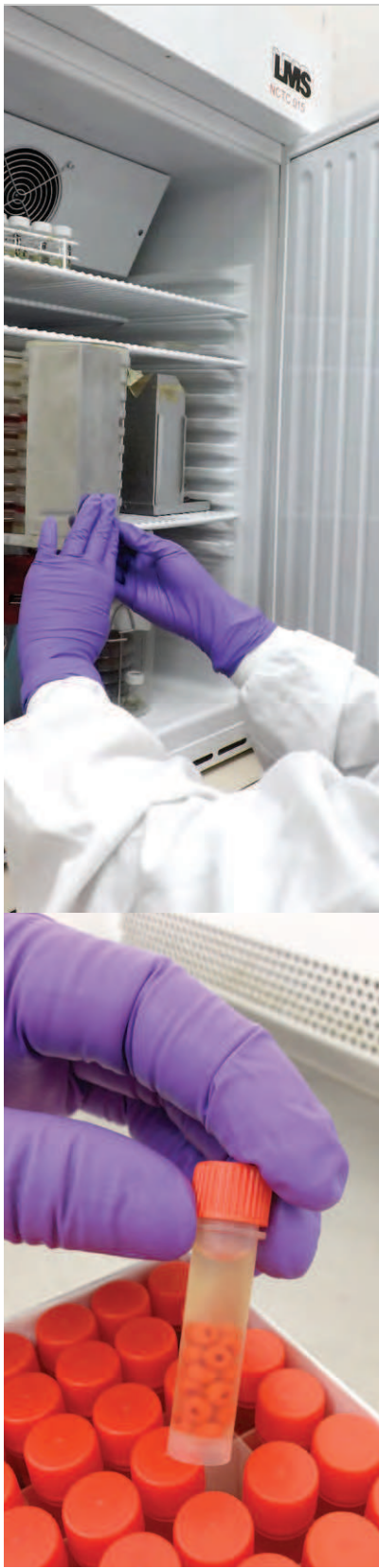
Many microbiology laboratories use NCTC authenticated cultures to produce their own stock cultures for daily or weekly use. This can be a cost effective means of maintaining control strains although it requires careful management and good attention to detail, particularly with recording the dates when the NCTC authenticated strain was reconstituted and subcultured, and when the stock cultures were made.

One of the most commonly used methods for creating stock cultures is to preserve bacterial strains as frozen cultures using commercially available beads in a cryopreservative broth. The beads are treated with a chemical that improves bacterial adhesion and different cryopreservative broths are available depending on the organism to be stored.

If laboratories store their stock cultures on beads it is important to consider the type of organisms to be preserved. This is because not all bacteria can be stored successfully on beads. Some strains are very fastidious in their requirements so need specially formulated cryoprotectants. Organisms in the same genus can vary in their suitability for storage on beads so laboratories are advised to consider the manufacturer's validation report to check which bacteria were used to validate the beads. It is also essential to check the manufacturer's instructions for preparing the stocks – this can differ depending on the product.

Some laboratories prepare stock cultures on agar slopes which can be suitable for short-term working stocks but this method is more challenging for long-term storage.

Important note:
Serially subculturing (passaging) bacteria can cause genetic variation so stock control strains should not be more than four subcultures from the NCTC authenticated strain. Laboratories are advised to record the number of subcultures. Checking the purity of stock cultures is paramount, regardless of whether they are stored on beads or slopes. Contaminated cultures or those that do not show a typical morphological appearance should be discarded and a new NCTC ampoule requested. It is never advisable to try to recover a control strain from a mixed culture.



Student placements at NCTC

NCTC supports scientists throughout their careers and is able to offer a limited number of placements as part of the PHE's work experience placement programme. The range of microorganisms included in the collection affords an excellent opportunity for students to learn about the diversity of bacteria of clinical and veterinary significance.

Laura Eshmene describes her year in NCTC:
Institution: University of Brighton, UK
Course: BSc Biomedical Science
Length of course: 4 years (including 1 year spent on placement)

What was your role at NCTC / what tasks did you carry out?: My role was primarily working on the quality control of new stocks of freeze-dried bacteria. This role included: identification tests, purity and viability checks and analysis on the MALDI-TOF MS and VITEK2.

How have you benefited?: The year spent working for NCTC has taught me valuable characteristics tests for a selection of their 5,000 strong collection of bacterial strains. I have increased confidence in carrying out laboratory work and have gained an excellent understanding of health and safety and test protocol. I was also able to complete my Evidence Portfolio for the Institute of Biomedical Science Certificate of Competence which, along with my year's laboratory experience, will improve my career prospects.

Highlights of the experience?: What I enjoyed particularly was working on my project; NCTC Cultures to Standardise Methods in Clinical Microbiology Testing Laboratories. This experience enabled me to learn and carry out many classical microbiology identification tests. I produced a poster for NCTC which was launched as a feature on the website. This project was an incredible opportunity and it's nice to know that the work I carried out was used to help laboratories not only in the UK but globally.

What did you learn/did anything surprise you/change how you see things?: I learnt so much about the routine work undertaken in PHE laboratories. I had many academic opportunities so I could attend conferences and seminars and therefore kept up-to-date with current health trends.

I was surprised at just how helpful everyone was who was involved in my training during the year. The knowledge in the Culture Collections team is overwhelming and I feel incredibly lucky to have been trained there.

How will your experience impact on your future career? As a result of this year my career prospects will have improved dramatically. I have a year's worth of experience working with some of the UK's best scientists, and I have grown as a person, with newfound qualities. My training this year has been wonderful and has set me up with knowledge and understanding of the work carried out as a biomedical scientist. I am thankful I have had and still have the support of the staff working for Culture Collections whose advice and training will help me in my career.

To download Laura's poster go to: www.phe-culturecollections.org.uk/nctc-strains-and-tp-poster

NCTC Cultures to Standardise Methods in Clinical Microbiology Testing Laboratories

L. Eshmene, H. McGregor, A. Deheer-Graham, R. Siddiqui, J. E. Russell
National Collection of Type Cultures, Culture Collections of Public Health England

INTRODUCTION

SMI TEST PROCEDURES USING NCTC STRAINS AS CONTROLS

FOR MORE INFORMATION

REFERENCES

ACKNOWLEDGEMENTS

NCTC - some frequently asked questions

Q How do I know which strains to use?

We provide an extensive online catalogue – go to:
www.phe-culturecollections.org.uk/products

Many peer-reviewed publications, internationally recognised methods, technical manuals and standard operating procedures will stipulate the use of NCTC cultures or provide general guidance. However, if you need additional advice you can contact us. NCTC microbiologists will be able to advise you or may refer you to another PHE expert in your particular field.

Tel: +44 (0)1980 612684
Email: culturecollections.technical@phe.gov.uk

Q How do I order my NCTC cultures?

You can order online using the Culture Collections website. Alternatively we can accept orders by email; you can download an order form from our website.

Q How are NCTC cultures dispatched?

NCTC cultures are sent by courier. Normally you receive your cultures within two to five days of dispatch. Cultures are sent as Biological Substances, Category B (UN3373) or Category A, Infectious Substances (UN2814 or UN2900), as appropriate. Export restrictions apply for the supply of hazardous pathogens.

Q What information is provided with my NCTC cultures?

Instructions for handling are provided with every NCTC order. A material safety data sheet (MSDS) and certificate of analysis are available from the website.

Q What is the difference between NCTC and ATCC cultures?

There are over 500 culture collections around the world containing microorganisms of significance in a range of different fields. ATCC refers to the American Type Culture Collection which is a large generalised culture collection. Type cultures are always deposited in more than one collection so there is some overlap between ATCC and NCTC strains. For example, NCTC strain 9001 (*Escherichia coli*) is the same strain as ATCC 11775. Our website provides further guidance on cross-referencing equivalent strains.

Q My laboratory subcultures NCTC strains to beads and stores the microorganisms in a freezer for use over time. Is there a problem with this procedure?

Problems can arise because strains that are repeatedly subcultured may lose their microbial integrity and traceability to the initial NCTC stock. Refer to page 24 for guidance on making your own stock cultures.

Many thanks to everyone who contributed to the content and images including:
Dr Matthew Hannah – Lead electron microscopist, Virus Reference Department, PHE
Dr Barry Holmes – Microbiology taxonomist, retired Head of NCTC
Zak Prior – Microbiologist, PHE
Dr Jimmy Walker – Water system microbiology and decontamination expert, PHE
Professor Neil Woodford - Head, Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI), PHE.
Anna Dumitriu – Artist whose work blurs the boundaries between art and science with a strong interest in microbiology and the ethical issues raised by emerging technologies. Her installations, interventions and performances use a range of digital, biological and traditional media including live bacteria, robotics, interactive technologies, and textiles. See pages 17 and 19.

Selectrol® is a first generation derivative of original stock cultures obtained exclusively from the National Collection of Type Cultures (NCTC®) or National Collection of Pathogenic Fungi (NCPF®). Where appropriate other international strain designations, such as the ATCC®, are listed for reference purposes only.