



Department  
for Environment  
Food & Rural Affairs

*The use of culture collections for the provision  
of reference standards: the cornerstone to  
accurate bacterial diagnosis*

# The National Collection of Plant Pathogenic Bacteria

Andy Aspin, Curator

**Fera**

# NCPPB Aims

To provide scientific support to the UK Plant Health Service and to maintain, for use by research and educational establishments, and by industry, cultures of the world's bacterial plant pathogens. It is intended that sufficient cultures shall be kept of each species to be representative of its geographic and host range, and of the variation within it.

# Why Use Culture Collections?

Repetitive sub-culturing can lead to:

- Attenuation of properties such as
  - Colony morphology
  - Pathogenicity
- Mislabelling
- Contamination



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Culture collections aim to provide strains that are:

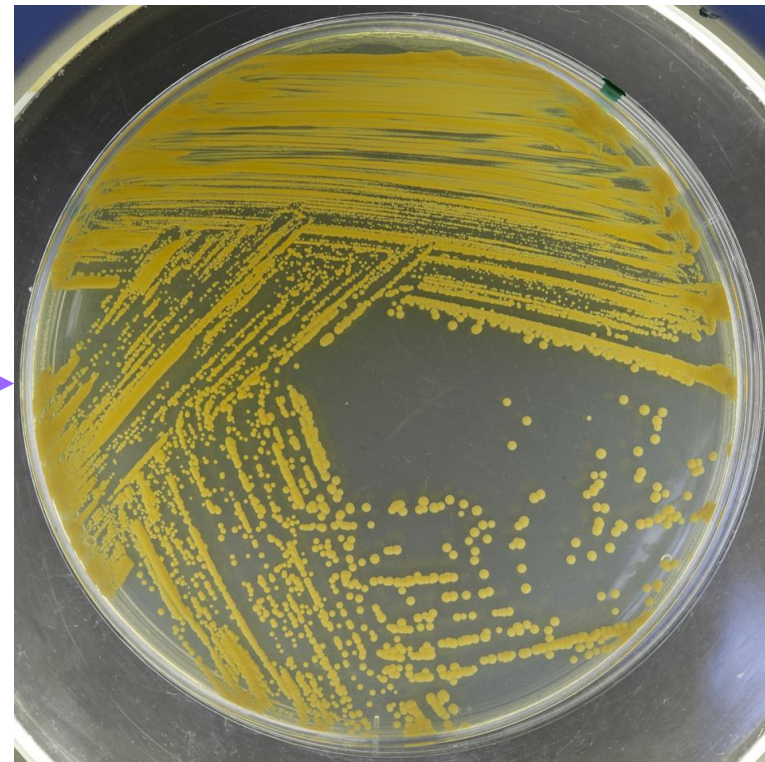
- As close to the original isolate as possible
- Pure and properly identified



# Reference Material From NCPPB Strains at Fera



Ampoule from  
the NCPPB  
*Source  
material*

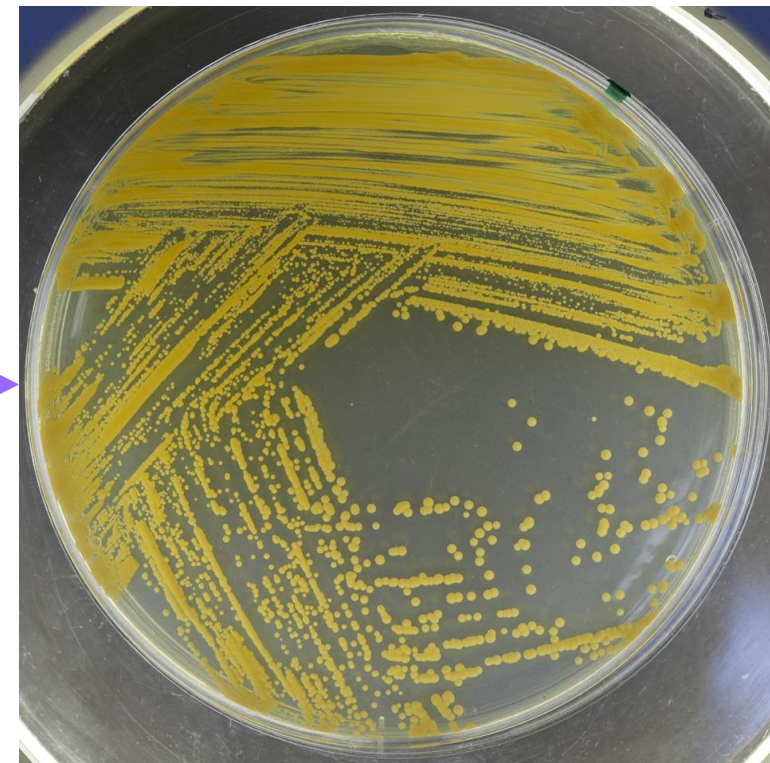


Raised on an agar  
plate

# Reference Material From NCPPB Strains at Fera



Ampoule from  
the NCPPB  
*Source  
material*



Raised on an agar  
plate

Stored on agar slope at 4°C  
*Reference stock*



Stored in glycerol at -80°C  
*Reference stock*



# Reference Material From NCPPB Strains at Fera

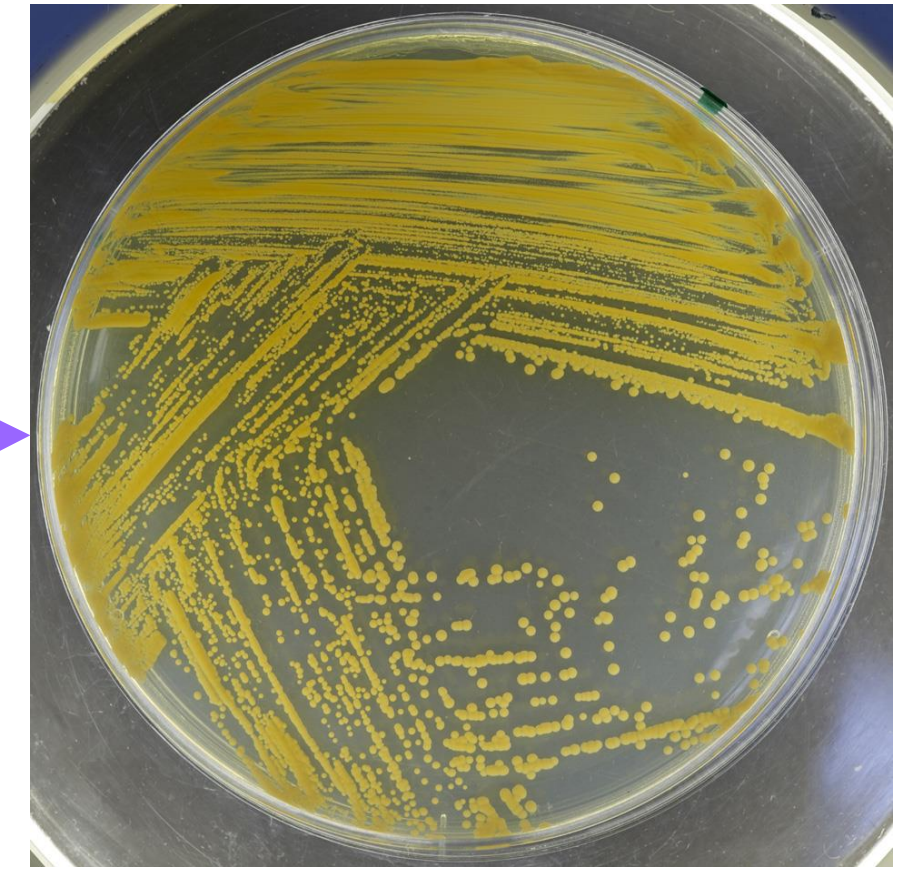


Ampoule from the NCPPB  
*Source material*



Raised on an agar  
plate

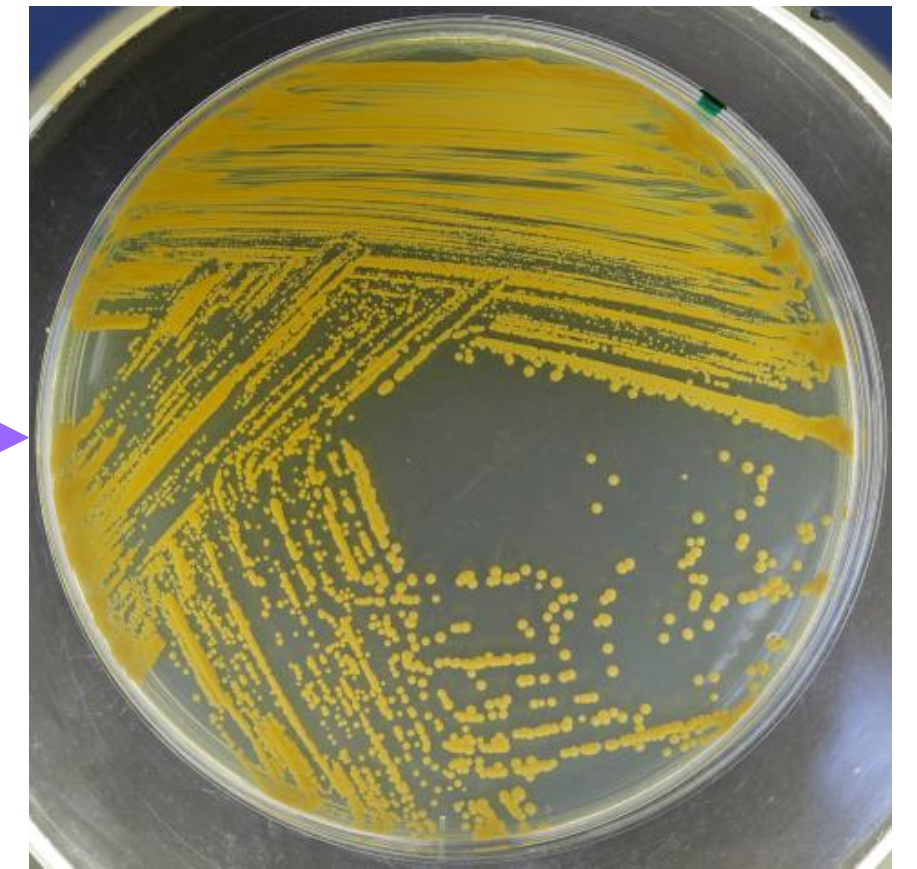
Stored on agar slope at 4°C  
*Reference stock*



Reference material



Stored in glycerol at -80°C  
*Reference stock*



# Guidelines and Standards

## WFCC



World Federation for Culture  
Collections publish guidelines for  
the *Establishment and Operation of  
Collections of Cultures of  
Microorganisms*

# Guidelines and Standards

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## ISO

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- *Guide 31*  
Reference materials —  
Contents of certificates,  
labels and accompanying  
documentation
- *17034*  
General requirements for the  
competence of reference  
material producers
- *Guide 35*  
Guidance for characterization  
and assessment of  
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# Guidelines and Standards

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## EPPO

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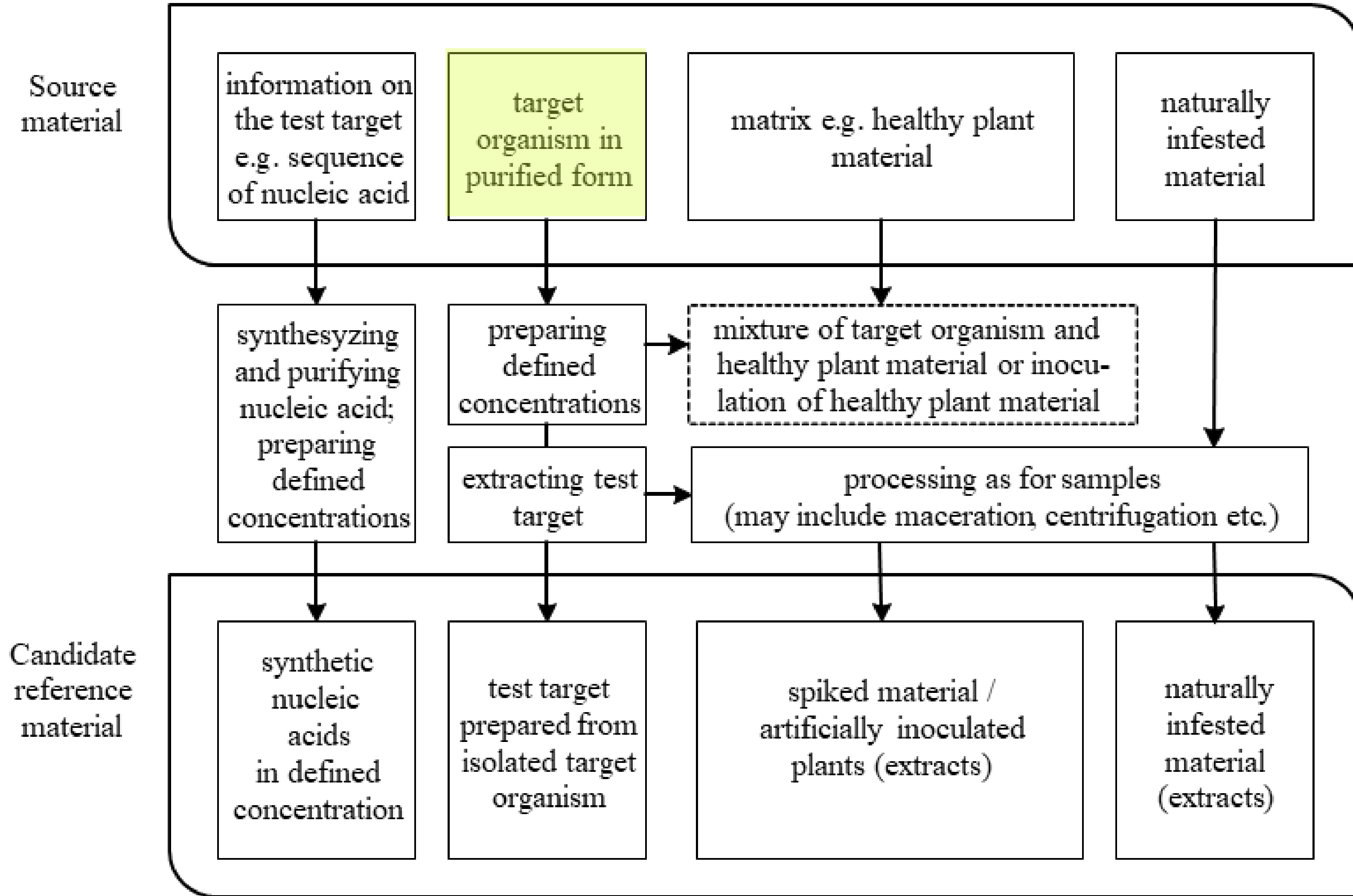
EPPO Standard PM7/147  
*Guidelines for the production of biological reference material*

*“the commercial availability of reference material or certified reference material is very limited and consequently reference material often needs to be produced by individual diagnostic laboratories.”*

*“well-defined and characterized reference material” “essential for internal and external quality checks, validation and verification of tests (including morphological identification) and interlaboratory comparisons.”*

# Descriptors of Biological Reference Material PM7/147

- Intended use of biological reference material;  
e.g. quality checks, validation, verification; target organism; limitations of use
- Type of material/commutability;  
degree of similarity between reference material and natural material
- Identity;  
Taxonomic value determined through at least two biological principles
- Traceability;  
When, where, by whom collected material; or a strain identifier in a collection
- Purity;  
Amount of the target organism's presence in the reference material
- Homogeneity;  
Degree of the organism's presence across different units of the reference material
- Stability;  
Degree at which the material will degrade over time
- Assigned values  
Qualitative or quantitative – possibly with a measure of uncertainty



Source material

information on the test target  
e.g. sequence of nucleic acid

target organism in purified form

NCPPB 909 *Ralstonia solanacearum* Brown rot  
NCPPB 2140 *Clavibacter sepedonicus* Ring rot

synthesizing and purifying nucleic acid; preparing defined concentrations

preparing defined concentrations

mixture of target organism and healthy plant material or inoculation of healthy plant material

extracting test target

processing as for samples (may include maceration, centrifugation etc.)

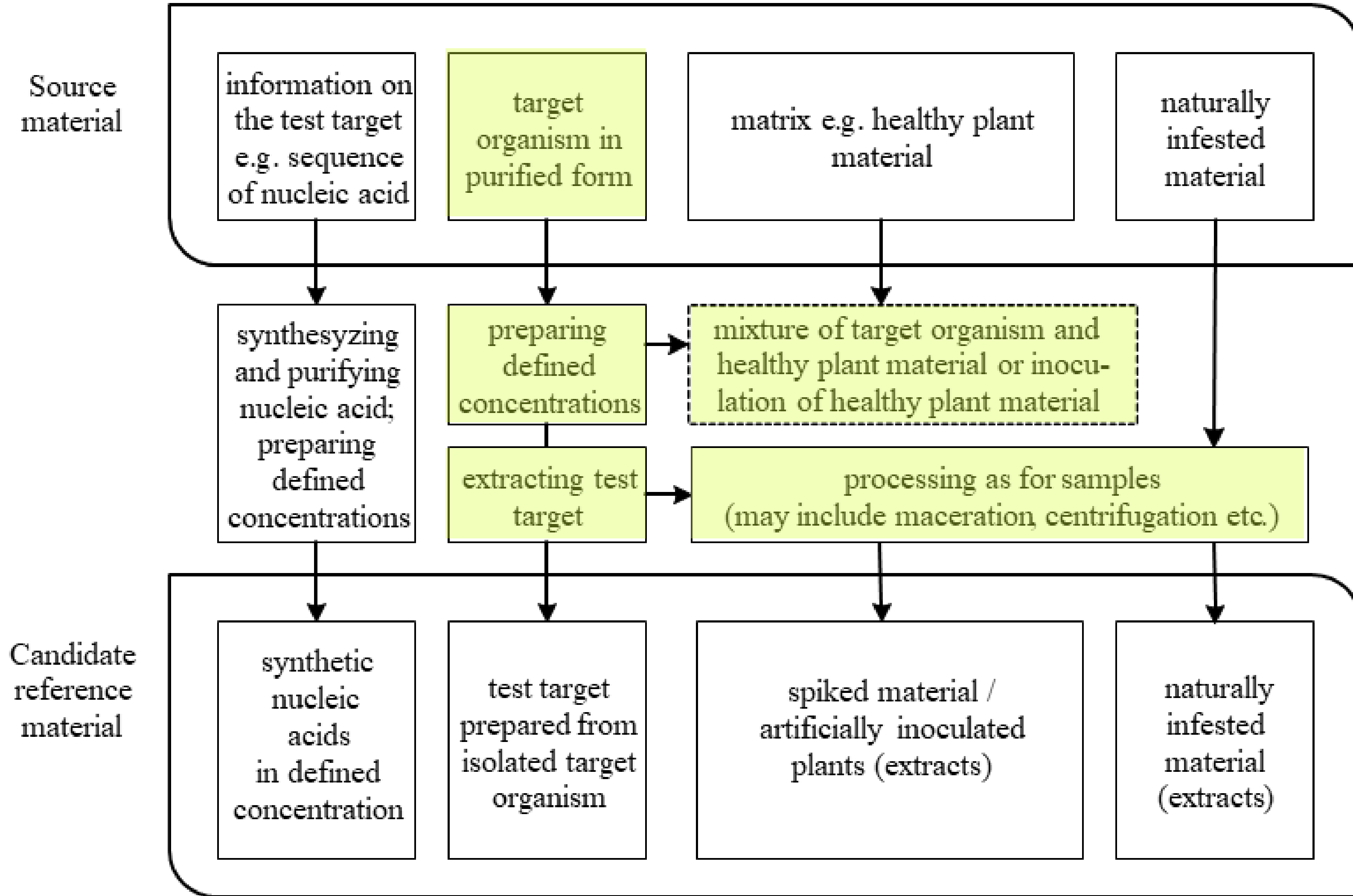
Candidate reference material

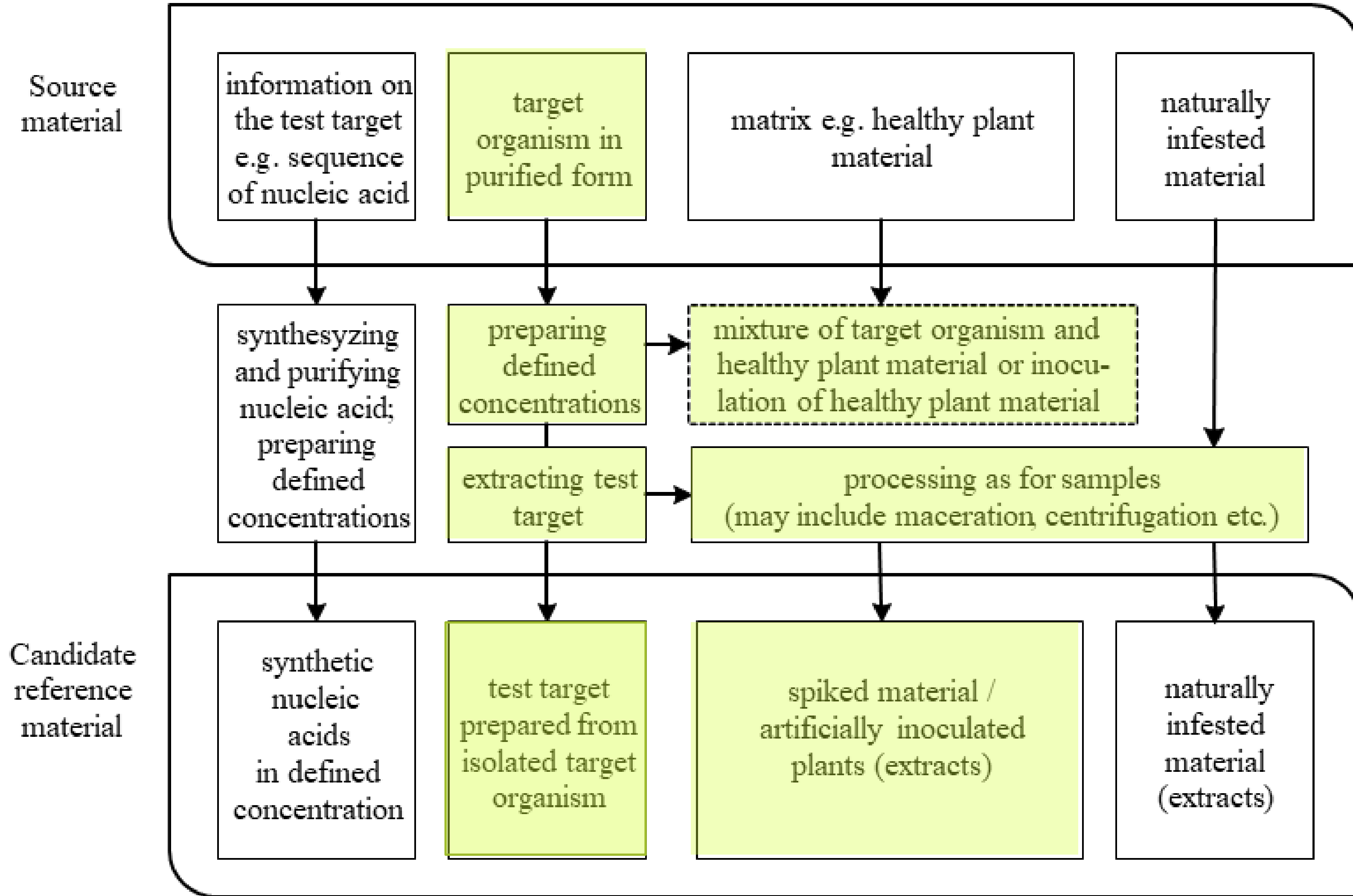
synthetic nucleic acids in defined concentration

test target prepared from isolated target organism

spiked material / artificially inoculated plants (extracts)

naturally infested material (extracts)





# Descriptors of Biological Reference Material

- Intended use of biological reference material;  
Interlaboratory comparison (Fera and SASA)
- Type of material/commutability;  
'Real' negative sample matrix spiked with cultured cells
- Identity;  
Strains characterised using IF, conventional PCR, real-time PCR, barcoding and MALDI-ToF
- Traceability;  
NCPPB 909 isolated 1961, Egypt, Ron Lelliott; NCPPB 2140 isolated 1942, USA, L.T. Richardson
- Purity;  
Only the target organisms are present in their respective samples
- Homogeneity;  
NCPPB 909 and NCPPB 2140 are present at  $10^6$  cfu/ml
- Stability;  
Material has been verified stable for 24 hours at 4°C
- Assigned values  
Spiked samples are either positive or negative for the two target organisms

# NCPPB History

## Funding Bodies

UK Government

Cambridge University

MAFF

Defra



**1880** *Erwinia amylovora* first isolated and described by T.J. Burrill in IL, USA 1882

**1920's** Bacterial plant pathogen isolates start being collected in the UK

**1930's** W. J. Dowson starts collecting bacteria

**1947** NCTC donates plant pathogenic bacteria to Dowson

**1956** Moved to purpose-built Plant Pathology Laboratory, is recognised as a 'National Collection', and R. A. Lelliott becomes the first curator of the NCPPB

**1980's** D. E. Stead becomes curator

**1996** The collection moves again to its present location in York

**2008** A. Aspin is curator

**2026** Present holdings ~ 4000 strains

# Accession and Pre Freeze-Drying Checks



NCPBPB (04GA04)  
Fera Science Ltd.  
York Biotech Campus  
Sand Hutton  
York  
YO41 1LZ  
United Kingdom  
Tel: + 44 (0)1904 462344  
Email: [ncppb@fera.co.uk](mailto:ncppb@fera.co.uk)  
Website: [www.fera.co.uk/ncppb](http://www.fera.co.uk/ncppb)

For use by NCPBPB  
Accession No.:  
Date received:

## Deposition Form

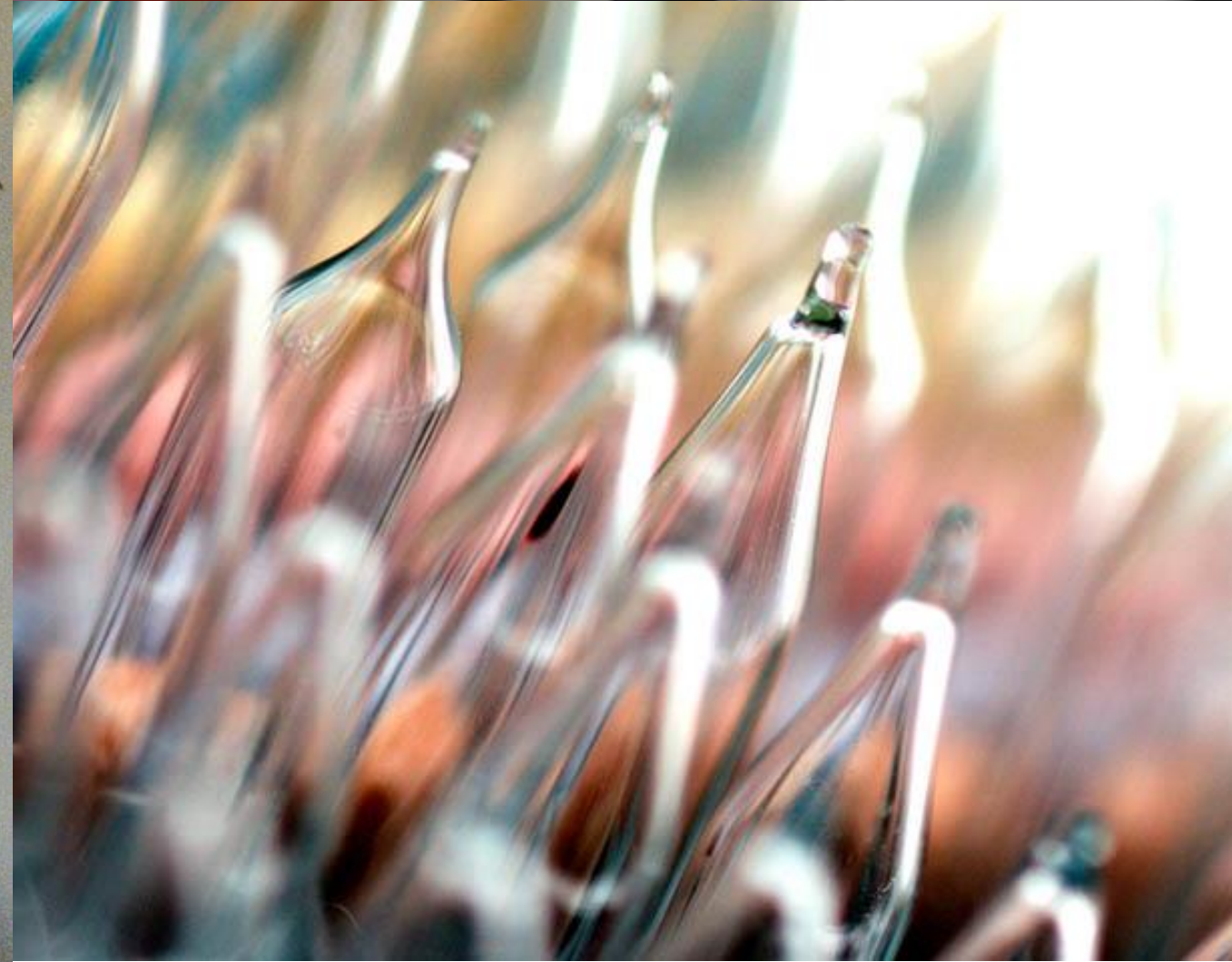
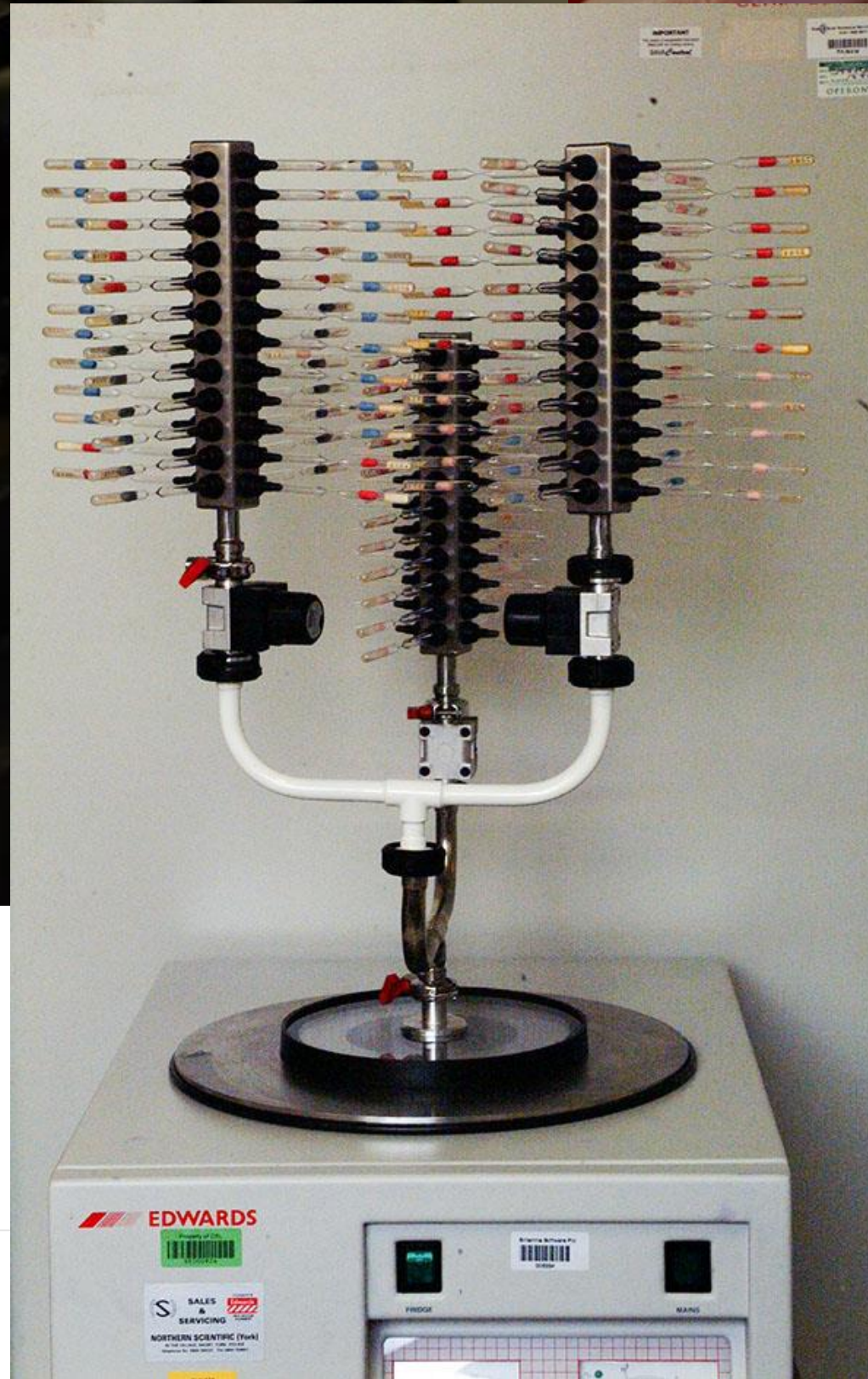
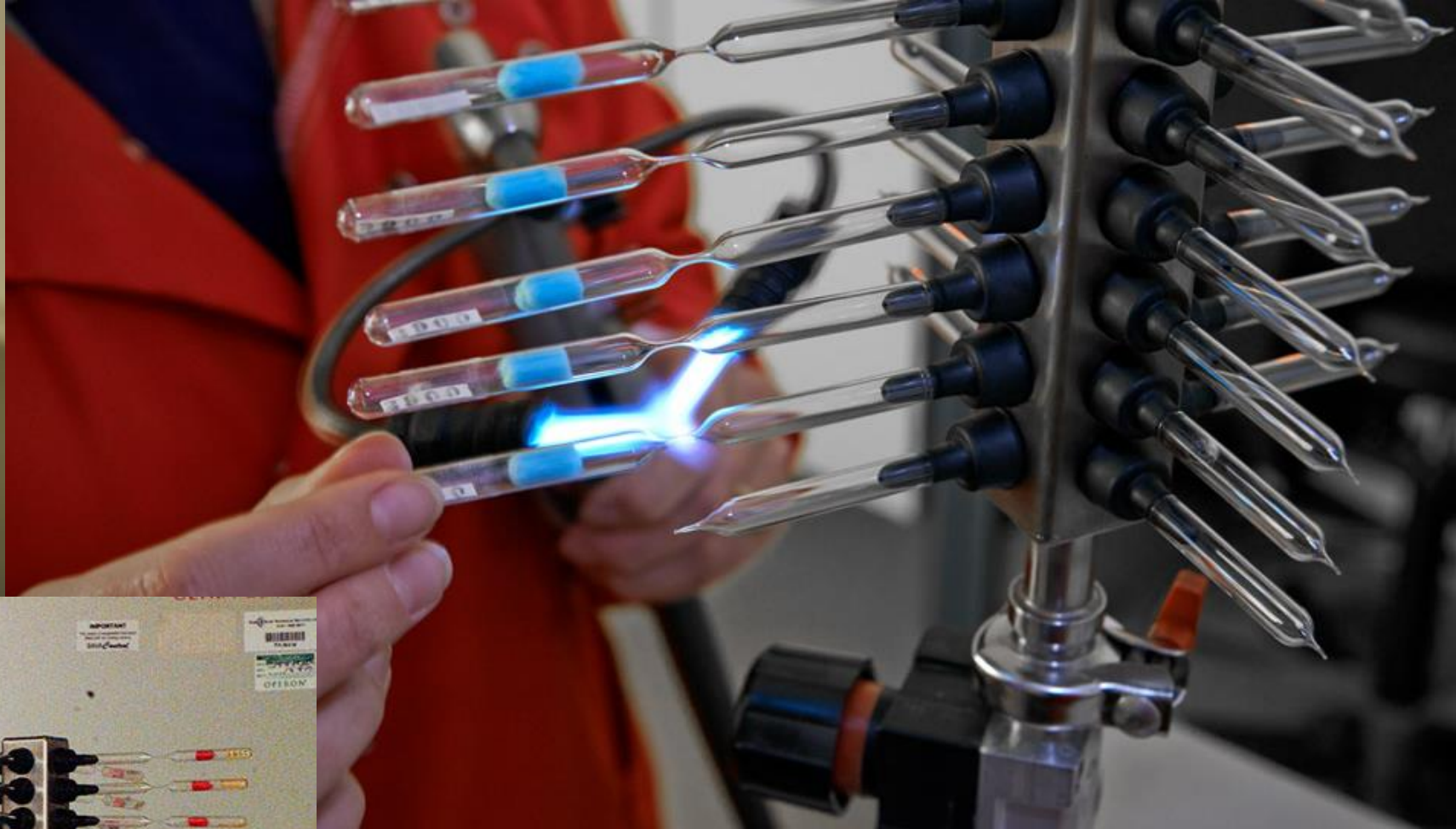
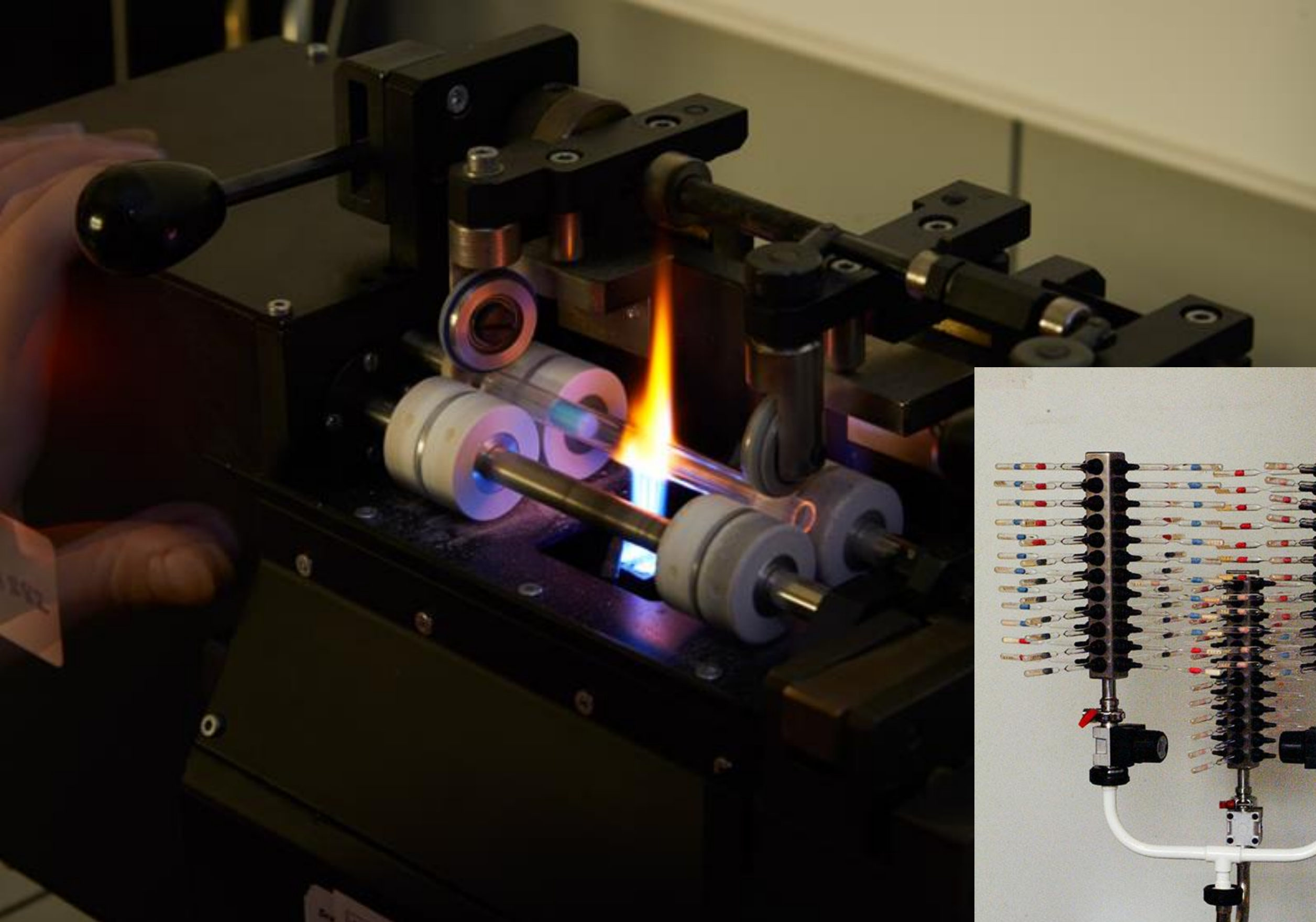
### Section 1 - Strain information

1	Name of organism: (genus and species) Authority: (In full, especially if a newly described taxon)	
2	Name of donor: Donor's culture reference number:	
3	History of culture: Individuals and/or institutions who isolated/maintained the culture prior to the donor. Please include their culture references.  Culture deposited in the NCPBPB ← ← ← ← ←	
4	Individual responsible for isolation of culture: The isolater's reference number:	
5	Host from which isolated (scientific & common name):	
6	Is the culture a type, cotype, neotype or reference strain (or being suggested as such)? Please give a reference if possible:	

## Deposition Form

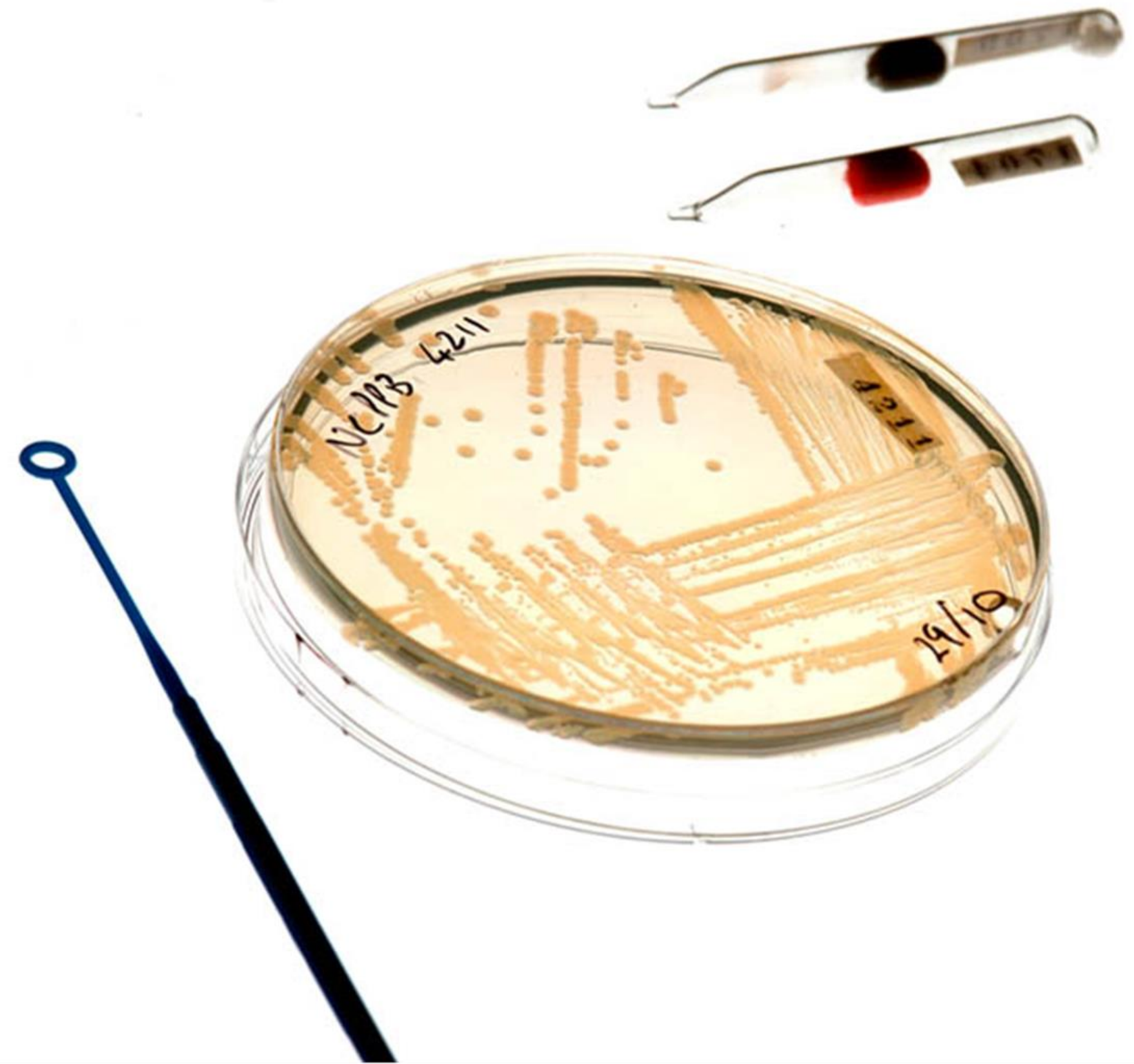
Any of the following identity checks:

- Colony morphology
- PCR and real time PCR
- Fatty acid profiling (ISO 17025)
- MALDI ToF
- Partial gene sequencing (barcodes)
- Whole genome sequencing



# Post Freeze-Drying Checks

- Purity
  - 10 days on Nutrient Agar – checking for gross contamination
- Authenticity
  - Same as the pre-freeze-drying checks
- Viability
  - Dilution counts to give an approximation of half life





# NCPPB in EPPO Standards

- 76 NCPPB strains are cited in 21 EPPO Standards
  - 37 Type strains
  - 9 Pathotype strains
  - 30 other strains

# Other Sources of Plant Pathogenic Bacteria

- Referenced in EPPO Standards

BCCM/LMG - Belgium

CECT - Spain

\*CIRM-**CFBP** - France

\*DSMZ - Germany

**NCPPB** - UK

- Other collections

ATCC - USA

**ICMP** - New Zealand

\* European registered collections for Access and Benefit Sharing regulations (Nagoya Protocol)

# NCPPB Team



Brian Carter  
Bacteriology  
Team Manager



Syed Mustafa  
Day to day management  
of the collection; author of  
the NCPPB MALDI-ToF  
library



Meyah Brown  
Maintenance of  
the collection



Cerys Evans  
Maintenance of  
the collection



Andy Aspin  
Curator



**Fera**