

Establishment of EUTCD-grade human embryonic stem cell banks for use as starting-material in clinical therapies

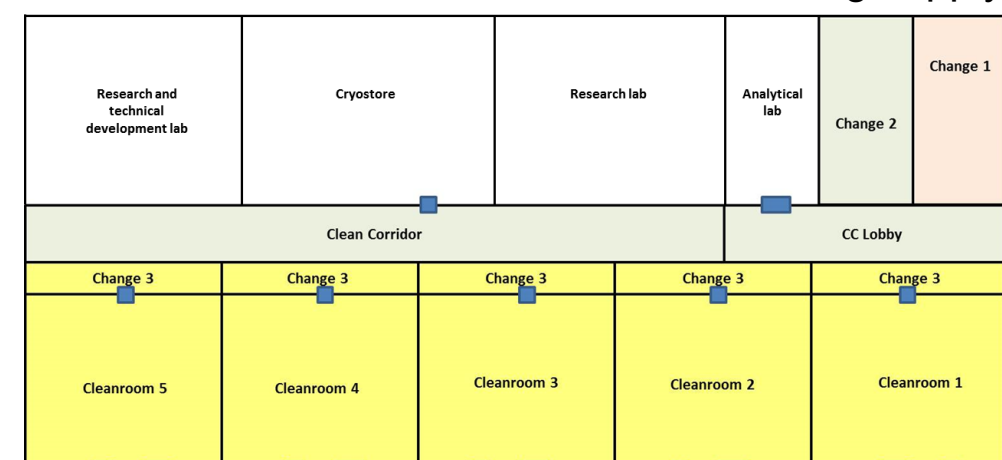
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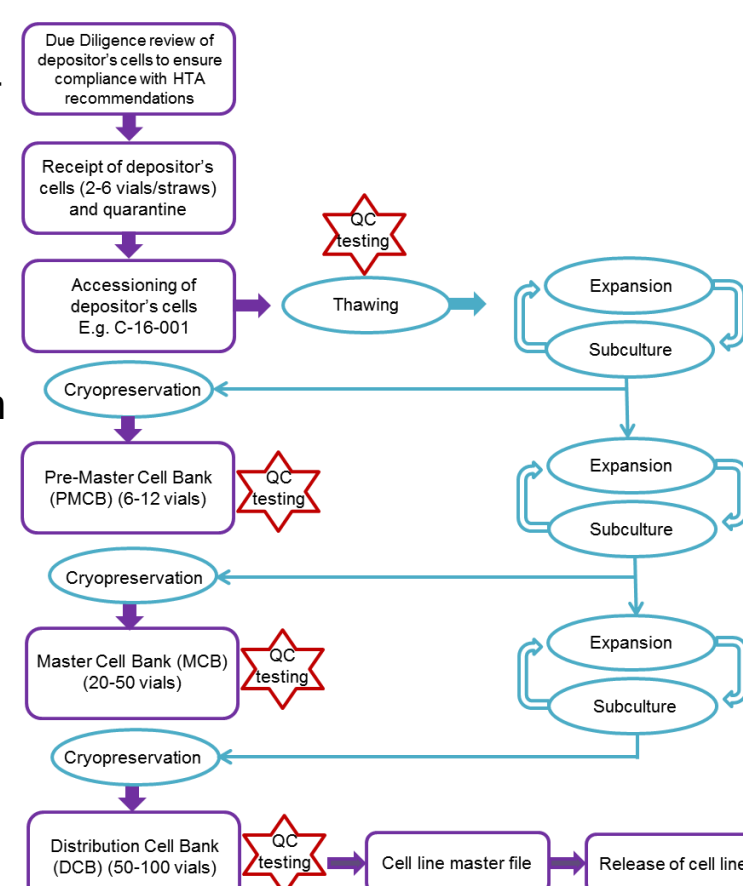
Overview of the Cell Banking Process

EUTCD-grade cell lines are cultured and banked at the UKSCB under the European Union Tissue and Cells Directives (2004/23/EC, (EU) 2015/565, (EU) 2015/566, 2006/17/EC and 2006/86/EC) which set a benchmark for the standards that must be met when carrying out any activity involving the use of human cells and tissues for therapeutic applications.

- Banking is an iterative process conducted in the UKSCB GMP compliant cleanrooms.
- Each cleanroom has its own air handling supply so acts as both a quarantine and production room.



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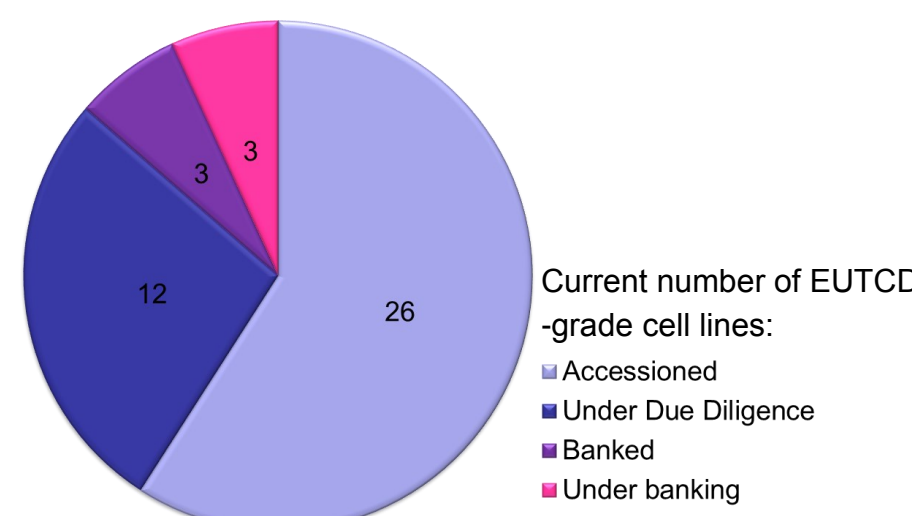
Due Diligence, Cell Receipt and Accessioning

Due Diligence Process

Cell lines proposed as EUTCD compliant undergo a due diligence process prior to Accession to ensure compliance of the cell line with the EUTCD as applied under HTA Directions. This is an important process, designed to assure that the cell line itself complies with the EU Tissue and Cell Directives required of cell lines used as starting materials in Advanced Therapy Medicinal Products (ATMPs)

Receipt of the cell line at the UKSCB

Cell sample integrity and depositor's documentation are checked upon receipt at the UKSCB to ensure the information matches the cells received.



Allocation of Accession Number

The accession number is a unique identifier used to track cells through the production process and is linked to the UK Steering Committee deposit reference number to ensure traceability. E.g. **C-17-001**. It is based on the “grade of cells” (Clinical, Research, Patent) and year (e.g. 2017) and number in year of deposit (e.g. 001). A unique Single European Code, Donation Identification Sequence is also allocated to each cell line as required in the EU.

Cell storage in quarantine liquid nitrogen LN₂ vessel

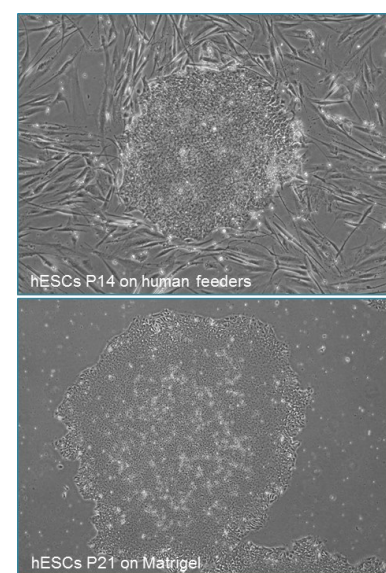
Following receipt and accessioning, EUTCD grade cell lines are stored in a separate LN₂ storage vessel until production commences.

Production

Culture and Cryopreservation of EUTCD grade Human Embryonic Stem Cells (hESCs)

- The pre-master cell bank, master cell bank and distribution cell bank are produced as a continuous process but may comprise discrete production runs
- Culture protocols are either taken from the depositor's protocols (UKSCB Cell Line Information Form) or standard UKSCB cell culture protocols are implemented
- EUTCD grade hESCs are:

- ⇒ Grown in serum-free, serum replacement, or xeno-free media with a formal risk assessment process applied to all critical reagents
- ⇒ Co-cultured on inactivated human feeders or synthetic matrices prior to banking
- ⇒ Passaged mechanically or using dissociation reagents (e.g. TrypLe™)
- ⇒ Banked using a controlled rate cooling method in cryogenic vials, as opposed to vitrification in straws
- ⇒ Placed into a dedicated “in-process” LN₂ storage vessel until QC testing is complete
- ⇒ The majority of frozen vials are stored at the UKSCB but a number of vials are also stored in a HTA compliant off site storage facility under a disaster recovery plan

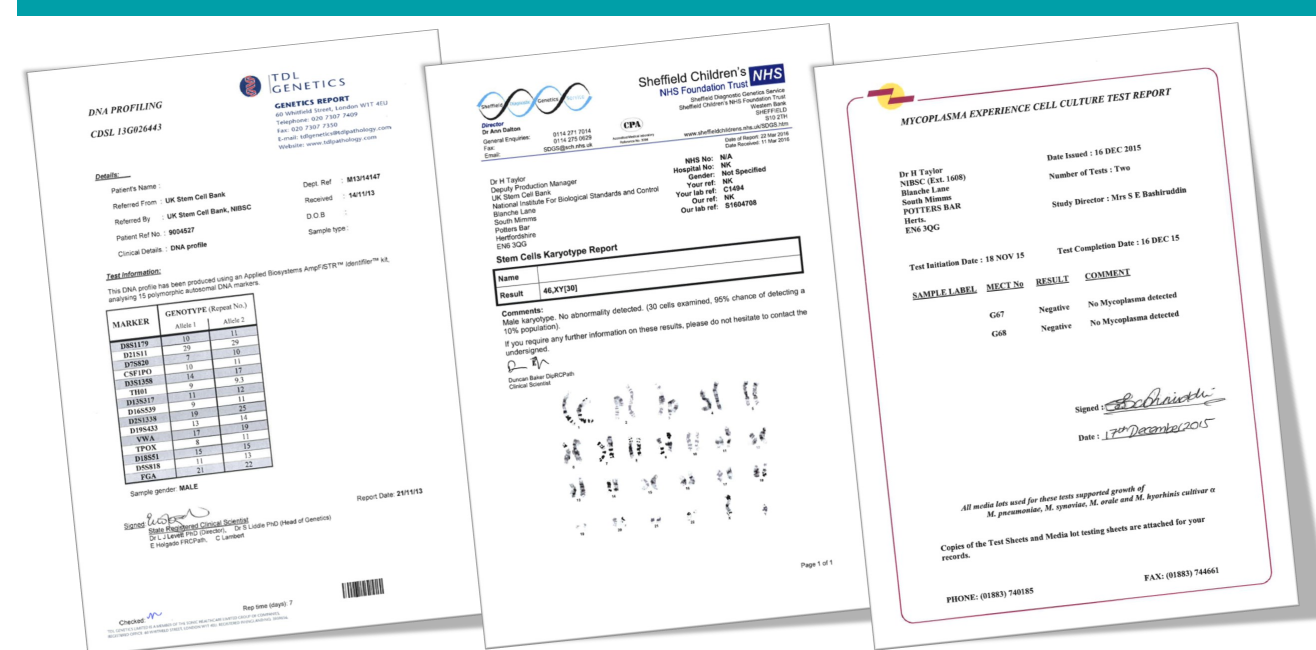


Quality Control Testing

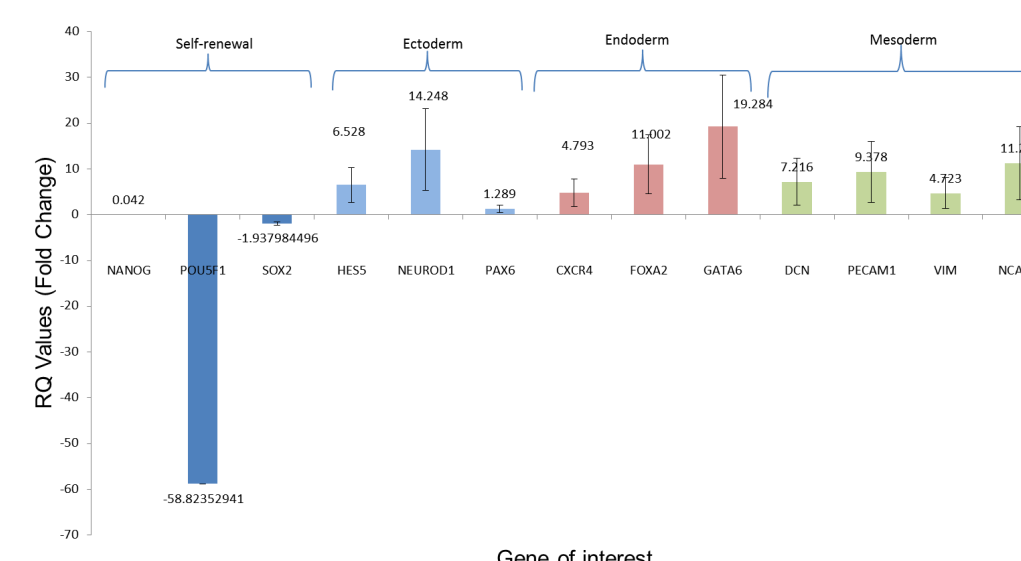
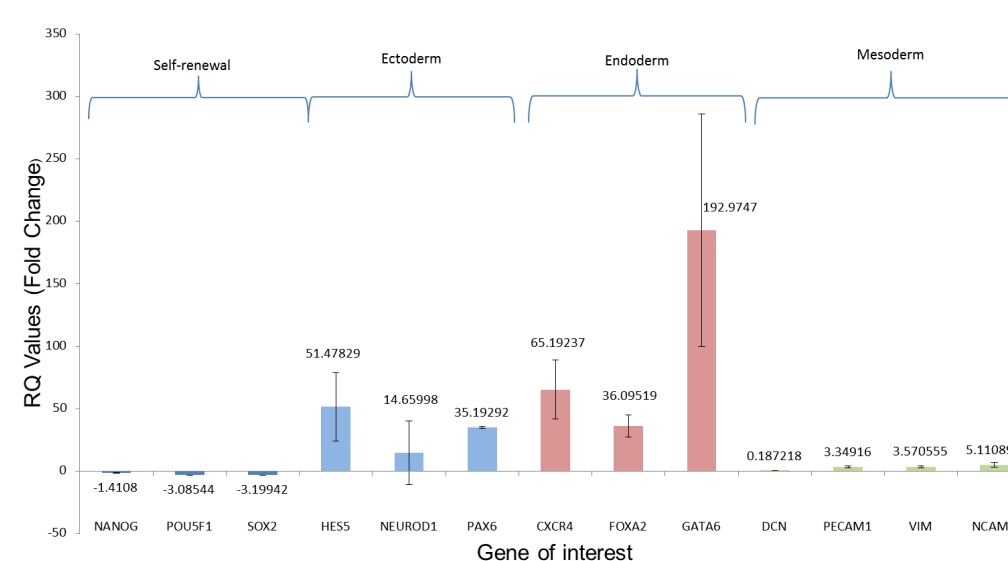
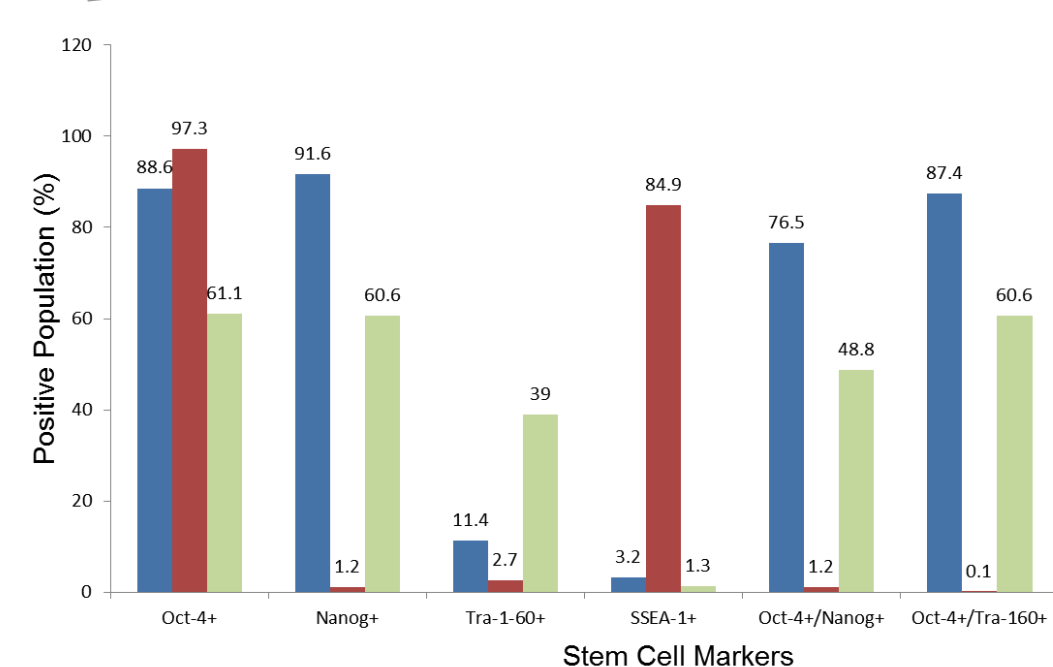
- During production of an EUTCD grade cell line, production records are completed and signed by the operator and reviewed by the Production Manager and Quality Assurance (QA) and consist of:

Mandatory Release QC test	Method
Viability	Trypan Blue Exclusion assay and NC3000
Sterility and in-process finger dabs and settle plates	Sterility: Cell supernatant incubated in Tryptone soya broth, Fluid thioglycollate medium and Sabaroid's liquid medium for 14 days; Finger dabs and settle plates are taken at the thawing and cryopreservation stages
Mycoplasma	EU Pharmacopoeial broth/culture test and Mycoplasma/Ureaplasma PCR
Viral PCR	DNA extract tested against a panel of human viruses: hCMV, HTLV1, HIV1, HIV2, HepB, HepC, EBV
DNA profiling	Applied Biosystems AmpF/STR Identifier kit
Informational test	Method
G-band karyotyping	Giemsa staining of metaphase chromosomes
"Stemness" markers	Flow cytometry analysis using the following panel of antibodies: Tra-1-60, Oct4, Nanog, and SSEA-1.
Differentiation	Embryoid body Q-PCR analysis of 13 self-renewal/ differentiation genes

Quality Control Testing



Example mandatory release test reports, from left: STR profile; G-banding karyology result; mycoplasma culture report.



Example informational test results, clockwise from top left: flow cytometry analysis to check for “stemness” markers; photomicrographs of embryoid bodies formed from hESCs to assess differentiation capacity; Q-PCR analysis of embryoid bodies to check for differentiation into the three germ layers (endoderm, ectoderm and mesoderm).

Cell Bank Release Procedure

- The Cell Line Master File** is firstly completed and reviewed:
 - The Cell Line Master File contains all information related to the cell line including traceability, production and QC documentation.
 - It is reviewed by UKSCB production, NIBSC Quality department and the Bank's designated individual prior to release of the cell line for distribution.
 - Ultimately it will feed into the Cell Line History File which will attribute to future Product Dossiers for regulatory review of stem cell derived cellular therapies
- Cell banks which “pass” QC release tests can then be moved to the “Release” LN₂ storage vessel
- The **UKSCB on-line catalogue** is updated to show that the cell line is now available: along with sex determination and any HLA data

http://www.nibsc.org/science_and_research/advanced_therapies/uk_stem_cell_bank/eutcd_grade.aspx

- Buyers will receive a vial of cells along with a **Certificate of Analysis** (see example below) and UKSCB protocols to assist with the thawing of the EUTCD-grade human embryonic stem cells.

National Institute for Biological Standards and Control

Certificate of Analysis **UK Stem Cell Bank**

Cell Line Name: CellLine123 Depositor: CellSupply123

UKSCB Accession No.: C-16-000 Cell Type: Human embryonic stem cell

UKSCB Reference No.: SCSC00-00 EUTCD Grade: ☒ Laboratory Grade: ☐

Intended Use: These cells are not for N/A No restrictions apply

Date Embryo Produced: 01/01/15 ☐ n/a Date Cell Line Derived: 01/03/15

Date Cell Bank Cryopreserved: 01/12/16 Date Cell Bank Released: 01/03/17 Expiry Date: 30 years from date of cryopreservation

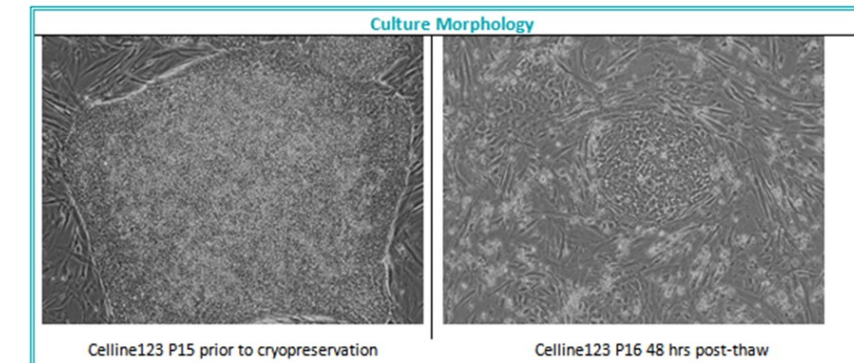
Bank Type: Distribution Bank ID No.: 001 Passage No.: P15 Cell Number / Vial: Nominal 1 x 10⁶

Feeder Dependent: ☒ Feeder Independent: ☐

Feeder Cell Line: Human (HDFn) Culture Matrix: Choose an item.

Recommended Culture/Storage Conditions: For feeder-dependent culture of Cell123, Nutristem media was used. Cultures should be incubated at 37°C and 5% CO₂ concentration. The cell line can be passaged both mechanically and/or with a dissociation reagent. Cells are cryopreserved at a cooling rate of 1°C/minute and stored at -196°C.

At 37°C for 5 mins and into 1 well of a 6-well plate Recommended Split Ratios for Established Cultures: 1:6 every 6 days

Culture Morphology: 

Additional Information: N/A

National Institute for Biological Standards and Control

Certificate of Analysis **UK Stem Cell Bank**

Biological Safety

Test Assay Acceptance Criteria Result

EP Sterility Bacteria/Fungi Not detected ☒ Pass ☐ Not Tested

Virus Panel Hepatitis B PCR Not Detected ☒ Pass ☐ Not Tested

Hepatitis C PCR Not Detected ☒ Pass ☐ Not Tested

HIV-1 PCR Not Detected ☒ Pass ☐ Not Tested

HIV-2 PCR Not Detected ☒ Pass ☐ Not Tested

Epstein Barr Virus (EBV) PCR Not Detected ☒ Pass ☐ Not Tested

Human Cytomegalovirus (CMV) PCR Not Detected ☒ Pass ☐ Not Tested

Viability and Identity

Test Assay Acceptance Criteria Result

Viability Trypan Blue 270K live cells prior to freezing ☒ Pass ☐ Not Tested

Cell Recovery Recovery and expansion of viable cells in culture after freeze/thaw Recovery of cells on thawing ☒ Pass ☐ Not Tested

Cell Line Identity STR analysis Data profile consistent with published profile or with profile obtained from pre-master cell bank material supplied by depositor ☒ Pass ☐ Not Tested

Informational Tests

Test Assay Acceptance Criteria Result

Morphology Visual assessment/photographic record Typical cellular morphology with low level of differentiation ☒ Pass ☐ Not Tested

G-Banding Expression of SSEA-1 Low ☒ Intermediate ☐ High ☐ Not Tested

Expression of Tra-1-60 Low ☒ Intermediate ☐ High ☐ Not Tested

Expression of Oct 4 Low ☒ Intermediate ☐ High ☐ Not Tested

Expression of Nanog Low ☒ Intermediate ☐ High ☐ Not Tested

Expression of Sox 2 Low ☒ Intermediate ☐ High ☐ Not Tested

Expression of SSEA-4 Low ☒ Intermediate ☐ High ☐ Not Tested

For all 'Tested' markers: Low <10% Intermediate 10-30% High >30%

For SSEA-4: Low <10% Intermediate 10-30% High >30%

Differentiation Potential Differentiation and qPCR for tri-lineage markers: Ectoderm: Detected Endoderm: Detected Mesoderm: Detected

HLA Information supplied by the Depositor LABtype: HLA-A, B, C, DRB1, DQA1 & DQB1

Release by: Date:

Approved by: Date:

For EUTCD-Grade cell lines ONLY

Release by: Date:

Acknowledgements

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