Medicines & Healthcare products Regulatory Agency

Non-Endotoxin Pyrogen Standards for the Monocyte Activation Test (MAT)

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MHRA overview

We are the Medicines and Healthcare products Regulatory Agency (MHRA)

We improve and protect the health of millions of people every day by making sure healthcare products in the UK **meet the highest standards** and are safe to use.

We are the UK regulator of medicines, medical devices and blood components for transfusion. We are responsible for making sure these products meet set standards for safety, quality and efficacy.

MHRA products and services

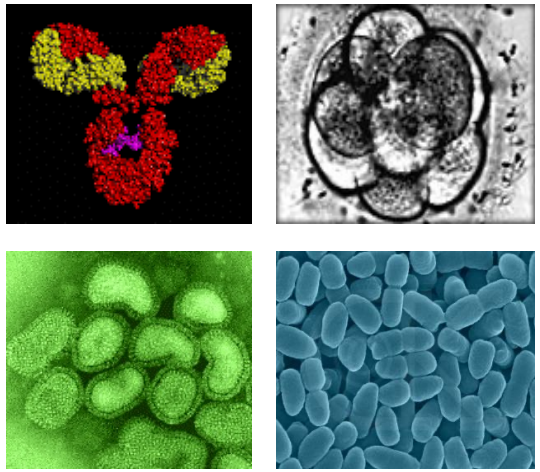
British Pharmacopoeia	The **British Pharmacopoeia** provides written and chemical quality standards for UK pharmaceutical substances and medicinal products.	MORE INFO
CPRD	**CPRD data services** provide the life sciences sector with anonymised primary care (NHS) datasets that can be linked to other datasets.	MORE INFO
NIBSC	**NIBSC standards** are available globally to set the quality of biological medicines. We also offer **NIBSC contract and control testing services**.	MORE INFO
UK Stem Cell Bank	The **UK Stem Cell Bank** offers quality-controlled stem cell lines to support scientific research and clinical development of stem cell therapies.	MORE INFO
Yellow Card	Our **Yellow Card scheme** is how patients, the public and healthcare professionals report problems with healthcare products.	MORE INFO

Why we need standards for biologicals

- Biological standardisation originated from a need to standardise variable bioassays that are used to measure the activity of **complex materials** that **cannot be completely defined** by other means

- For biologicals, identity and quantity determination are not enough – we also need to be able to (reliably) measure what something *does*

- Since it was originally demonstrated for insulin in the early 20th century, many studies have clearly shown the benefit of using a standard to express potency in ***relative terms*** for reliable batch-to-batch monitoring



NIBSC Standards

International Standards: WHO primary reference materials with defined unitage

e.g. 10/178 WHO International Standard 3rd IS for Endotoxin 10,000 IU/vial

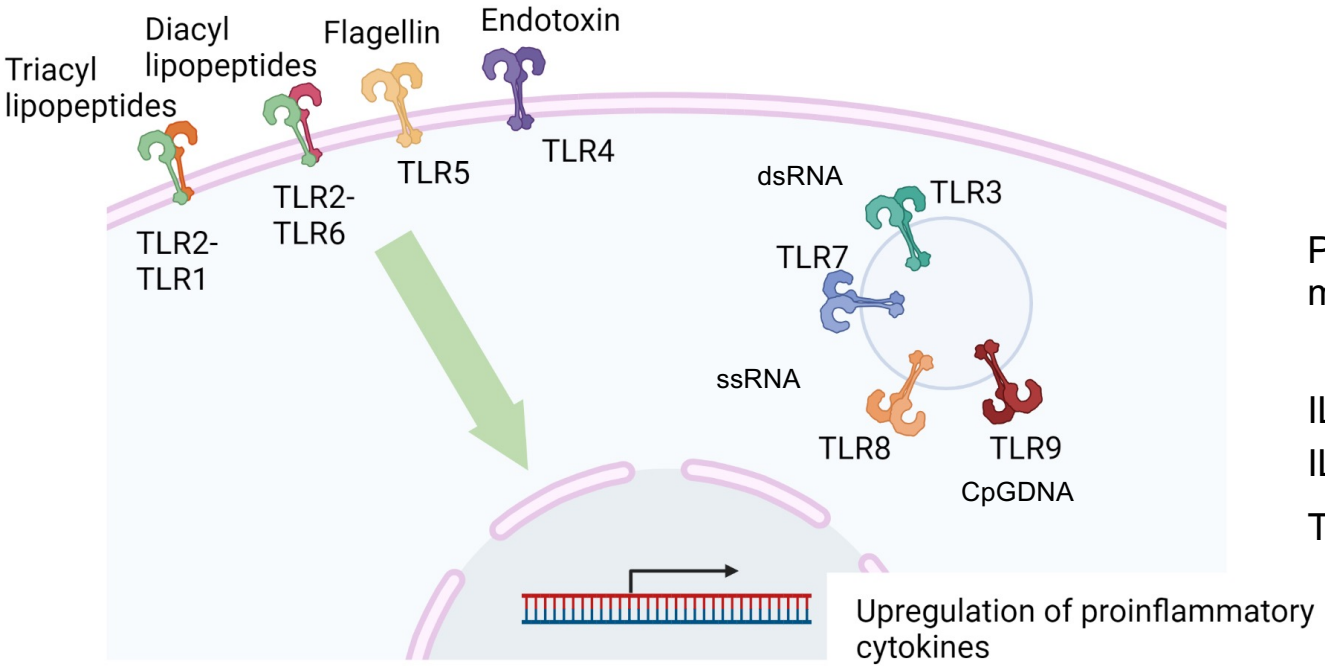
International Reference reagents: WHO reference material with arbitrary unitage assigned-interim reference material replaced by IS

Working Standards: validated against primary standards used in routine day-to-day testing (e.g Pharmacopoeia Standards)

Non-WHO Standards: materials which do not fall into the categories above

The status of a standard can change

Basis of the MAT



Pro-inflammatory cytokines quantified as a measure of pyrogenicity:

IL-1
IL-6
TNFα

All known to be involved in inducing fever

Monocyte Activation Test European Pharmacopoeia:

2.6.30. MONOCYTE-ACTIVATION TEST

6. PREPARATORY TESTING

To ensure the validity of the test, preparatory tests are conducted to verify that the criteria for the endotoxin standard curve are satisfied, that the test solution does not interfere with the test, that the test detects endotoxins and non-endotoxin contaminants and that the solution does not interfere in the detection system.

6-5. *METHOD VALIDATION FOR NON-ENDOTOXIN MONOCYTE-ACTIVATING CONTAMINANTS*

Preparatory testing is also to show that the chosen test system detects, in addition to bacterial endotoxins, non-endotoxin pro-inflammatory or pyrogenic contaminants. The suitability of the method for the particular product has to be verified. Preparatory testing is to include validation of the test system using at least 2 non-endotoxin ligands for PRRs, e.g. peptidoglycans, lipoteichoic acids, synthetic bacterial lipoproteins, flagellin and crude bacterial whole cell extract, at least 1 of which is to be spiked into the preparation to be examined. If available, historic batches found to be contaminated with non-endotoxin contaminants that caused positive responses in the rabbit pyrogen test or adverse drug reactions in humans should also be included.

Spike recovery must be within 50-200 per cent. However, if there is synergism between the PRR ligand and the test solution, it is sufficient for spike recovery to be greater than 50 per cent. The choice of non-endotoxin pyrogens used should reflect the most likely contaminant(s) of the preparation to be examined. The test system should ensure that at least TLR4 and 2 other TLR ligands that reflect the most likely contaminant(s) of the preparation tested are detected.

Monocyte Activation Test European Pharmacopoeia (2):

2.6.40. MONOCYTE-ACTIVATION TEST FOR VACCINES CONTAINING INHERENTLY PYROGENIC COMPONENTS

1. INTRODUCTION

This general chapter describes the use of the monocyte-activation test (MAT) to test vaccines containing inherently pyrogenic components (e.g. outer membrane vesicles, lipidated proteins).

The method described below can be used to monitor the consistency of pyrogen levels when the pyrogens in question are an integral part of the vaccine. The relevance of the MAT for this purpose is based on a risk assessment that takes into consideration the nature of the pyrogenic components, the magnitude of the pyrogenic activity and the control strategy applicable for these pyrogens (where appropriate, see the additional risk assessment factors outlined in general chapter *5.1.10*, section 3).

This general chapter is to be used in conjunction with general chapter *2.6.30. Monocyte-activation test*.

2. IN-HOUSE REFERENCE LOT

The in-house reference lot is a vaccine lot that has been found to be safe and efficacious through clinical studies, or is representative thereof.

on the known pyrogenic components of the vaccine to be tested. The receptor expression may be tested with specific ligands as described in section 6-1.

The cell line is cultured and maintained as described in chapter *2.6.30*.

6. PREPARATORY TESTING

To ensure the validity of the test, preparatory tests are conducted to establish that the test system is able to detect the pyrogenic components under consideration and that the test solution does not interfere in the detection system.

6-1. *METHOD VALIDATION FOR THE DETECTION OF INHERENTLY PYROGENIC COMPONENTS*

During assay development, the chosen test system must be shown to consistently detect the relevant pyrogenic components of the vaccine. The test system, including cryopreservation where applicable, may be validated using individual Toll-like receptor (TLR) ligands in addition to the reference lot to generate dose-response curves. Ligands could include: lipopolysaccharide (LPS) for TLR4 and non-endotoxin ligands such as lipoteichoic acid or peptidoglycan for TLR2, synthetic bacterial lipoprotein for TLR2-TLR1 or TLR2-TLR6, or flagellin for TLR5. Evaluation of the dose-response curves generated (using, for example, EC_{50}, limit of detection) allows the user to assess whether the cells are sufficiently sensitive in response to each ligand, as well as to gauge donor variability.

Standards for the MAT

3rd International Standard for Endotoxin
NIBSC code: 10/178

Contains 10,000 IU per vial

(Determined by BET/LAL)

TLR4 receptor

Can be used in MAT:

To qualify TLR4 response in cells

To quantify amount of endotoxin in a sample (product specific validation required)

To define an endotoxin equivalent value

New Non-Endotoxin Pyrogen standards– to stimulate TLRs other than TLR4

Project with EDQM in collaboration with PEI

Aim to produce 3 materials which stimulate 3 other TLRs

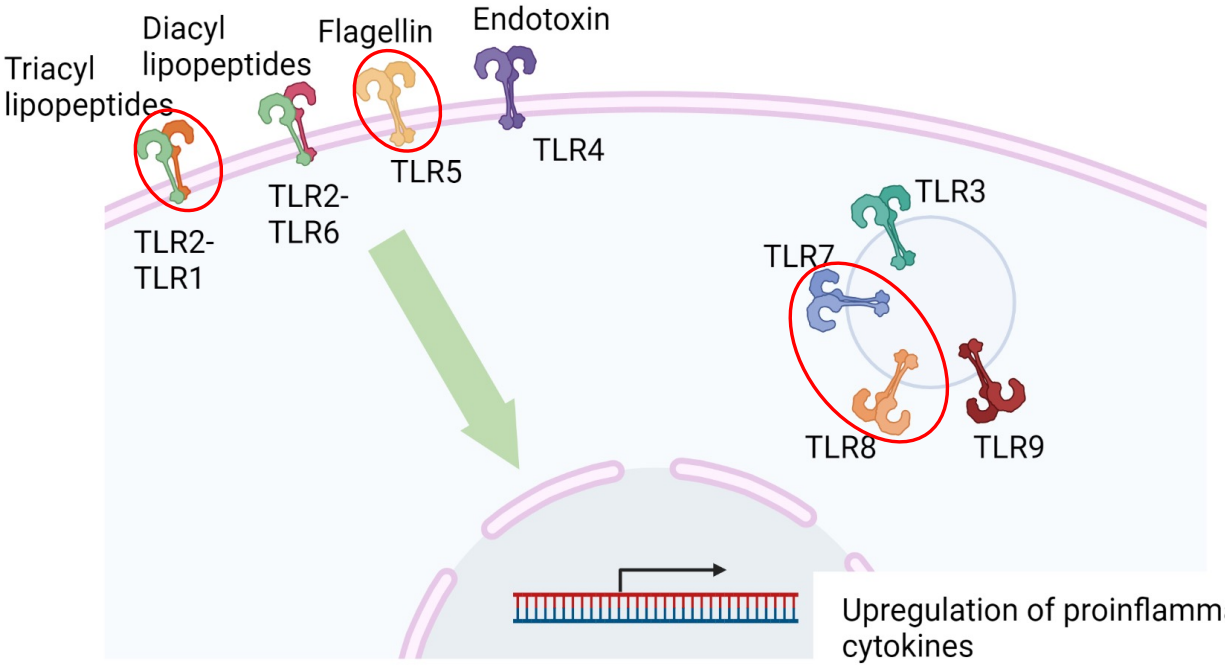
Use:

To qualify cells – positive control

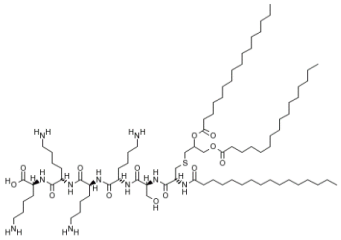
Will have no unitage

Will not provide a limit which equates to pyrogenic response

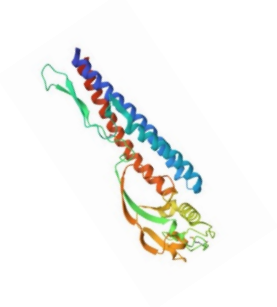
Reference materials under development



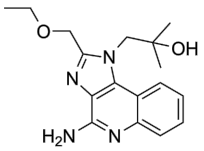
TLR2-TLR: Pam3CSK4
Pam3CysSerLys4
Synthetic triacylated lipopeptide



TLR5: FLA-ST
(*Salmonella typhimurium* flagellin)

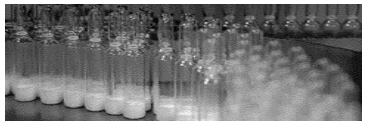


TLR7/TLR8 – R848
Resiquimod

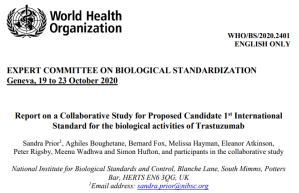


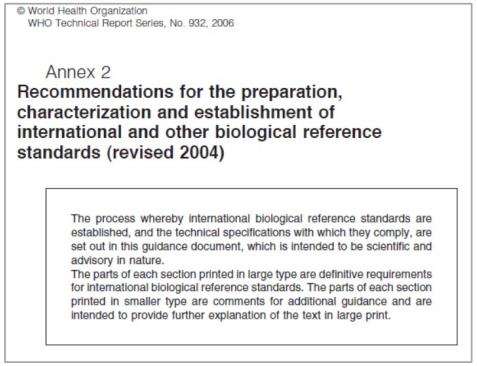
The development process for a WHO reference reagent

Reformulated & lyophilised



Evaluation of fitness for purpose (including commutability)





World Health Organization

Project endorsement (ECBS)

Sourcing Candidate material

Process development

Definitive (Large Scale) Fill*

Collaborative study

Study Report

WHO public consultation

World Health Organization

Establishment of WHO IS (ECBS)

Characterisation (Residual moisture, Head oxygen, appearance…)

Data from Participants → **Statistical analysis (MHRA)**

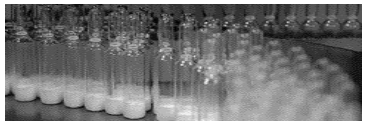
Stability (Real time and accelerated degradation studies)

Pam3CSK4 and R848

Definitive fills imminent

Collaborative studies - planning

Reformulated & lyophilised



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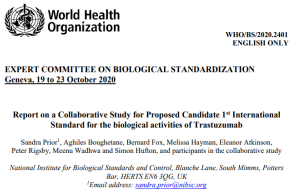


Project endorsement ✓

Sourcing Candidate material ✓

Process development ✓

Definitive (Large Scale) Fill*

Collaborative study

Study Report

WHO public consultation

Establishment of WHO IS (ECBS)

Endorsed by ECBS and EDQM

Commercially available-ease of replacement

Suitable formulation identified material stable tested in MAT and TLR specific cell lines

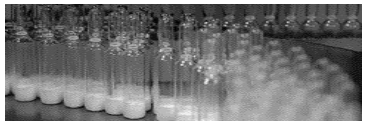
Characterisation (Residual moisture, Head oxygen, appearance…)

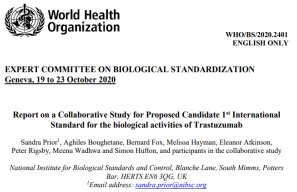
Data from Participants → Statistical analysis (MHRA)

Stability (Real time and accelerated degradation studies)

FLA-ST

Reformulated & lyophilised





World Health Organization

Project endorsement ✓

Endorsed by ECBS and EDQM

Sourcing Candidate material

Commercially available but issues with consistency

Process development

Definitive (Large Scale) Fill*

Characterisation (Residual moisture, Head oxygen, appearance…)

Evaluation of fitness for purpose (including commutability)

Collaborative study

Data from Participants

Statistical analysis (MHRA)

Study Report

WHO public consultation

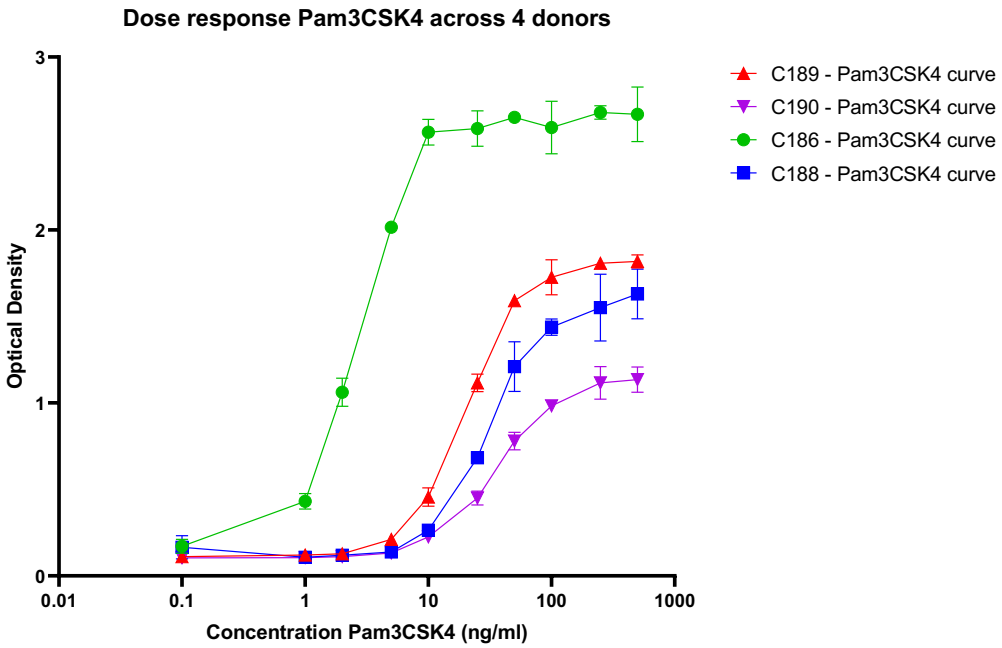
World Health Organization

Establishment of WHO IS (ECBS)

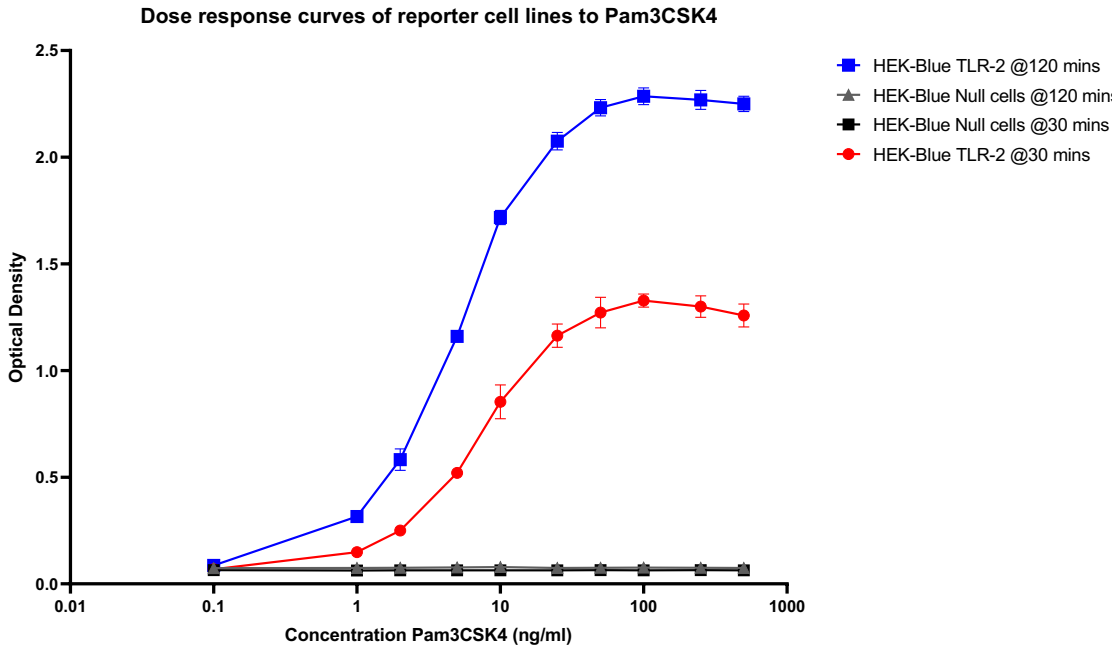
Stability (Real time and accelerated degradation studies)

Characterising the material-Pam3CSK4- also in BET

- Demonstration of dose response in PBMC assay

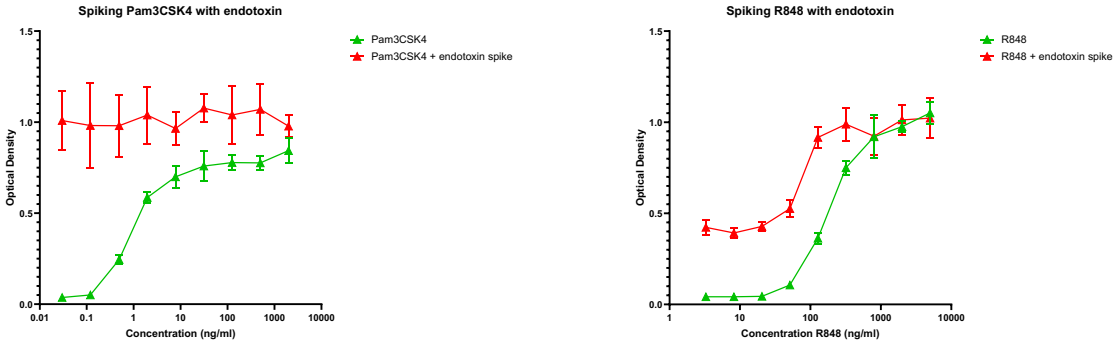


Demonstration of specificity using TLR reporter cell lines



Characterising the material

- LAL test for contaminating endotoxin- all negative
- Spiking with 0.5IU/ml endotoxin
- Comparison to endotoxin
- Estimate of IL-6 levels which are associated with fever



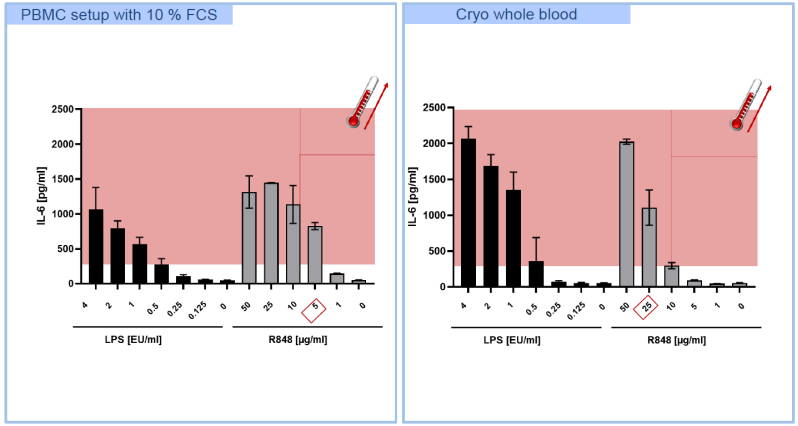


Figure courtesy PEI

Next Steps

- Collaborative study to assess fit-for-purpose Pam3CSK4 across the MAT assay formats
- Continue to develop TLR-5 material

- Contacts:
- For further information on NIBSC standards: standards@mhra.gov.uk
- To register interest in being part of collaborative study trusha.desai@mhra.gov.uk

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