# Solving problems with interpretation of PCR-ribotyping results:

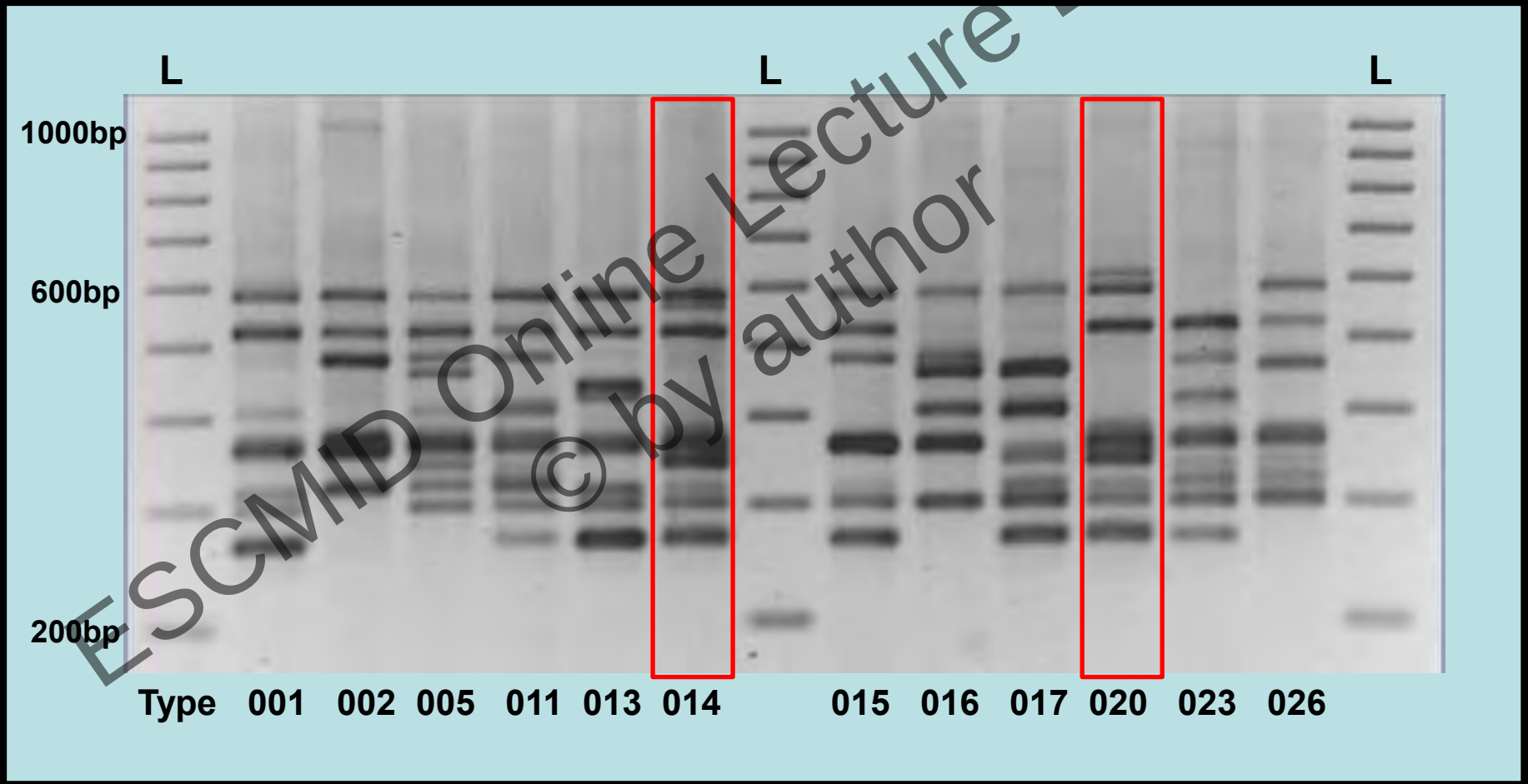
*general discussion*

**Professor Mark Wilcox**

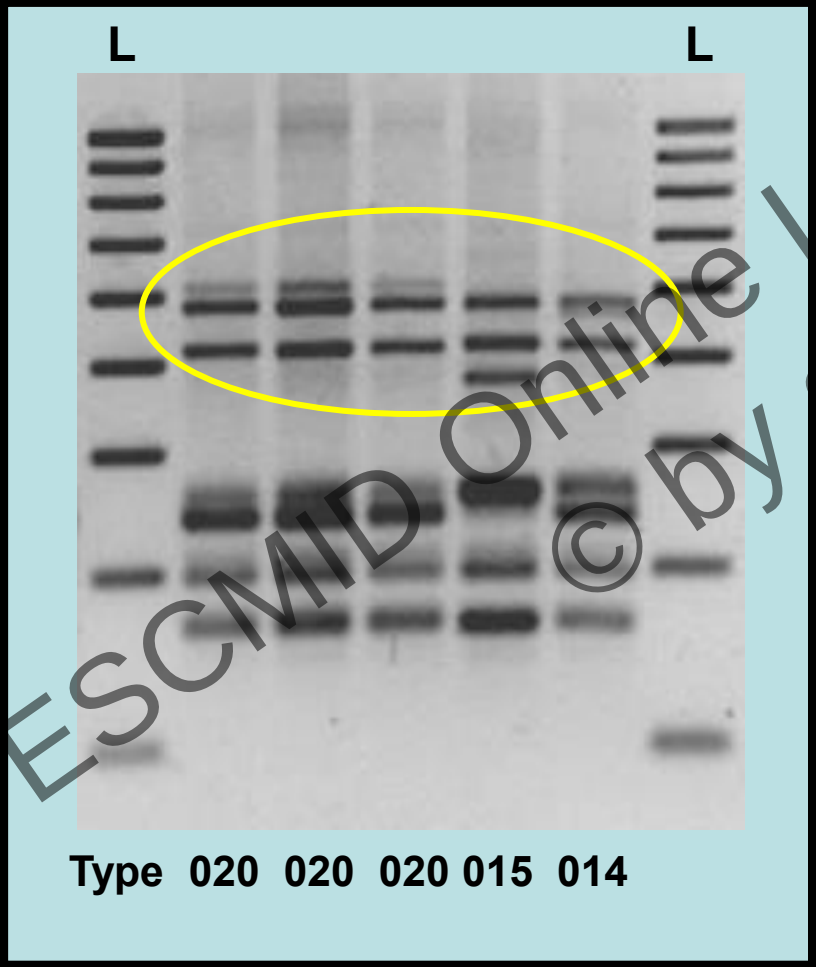
Leeds Teaching Hospitals, University of Leeds

& Public Health England, UK

# Examples of *C. difficile* ribotypes



# *C. difficile* PCR-ribotyping gel






Some types are very similar but may be reliably distinguished on good quality gels.

It is important to follow the method accurately and use the recommended primers, ladder, agarose, etc.

# Key points

- The original (Cardiff-based) ribotyping database was constructed using gel based ribotyping.
- It is clear that capillary-based ribotyping offers better resolution and consistency of banding patterns.
- There is a need to create an accessible ribotyping database; demand for this is increasing as more countries and laboratories are basing *C. difficile* typing surveillance on ribotyping.

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RESEARCH ARTICLE

# Development and Validation of an Internationally-Standardized, High-Resolution Capillary Gel-Based Electrophoresis PCR-Ribotyping Protocol for *Clostridium difficile*

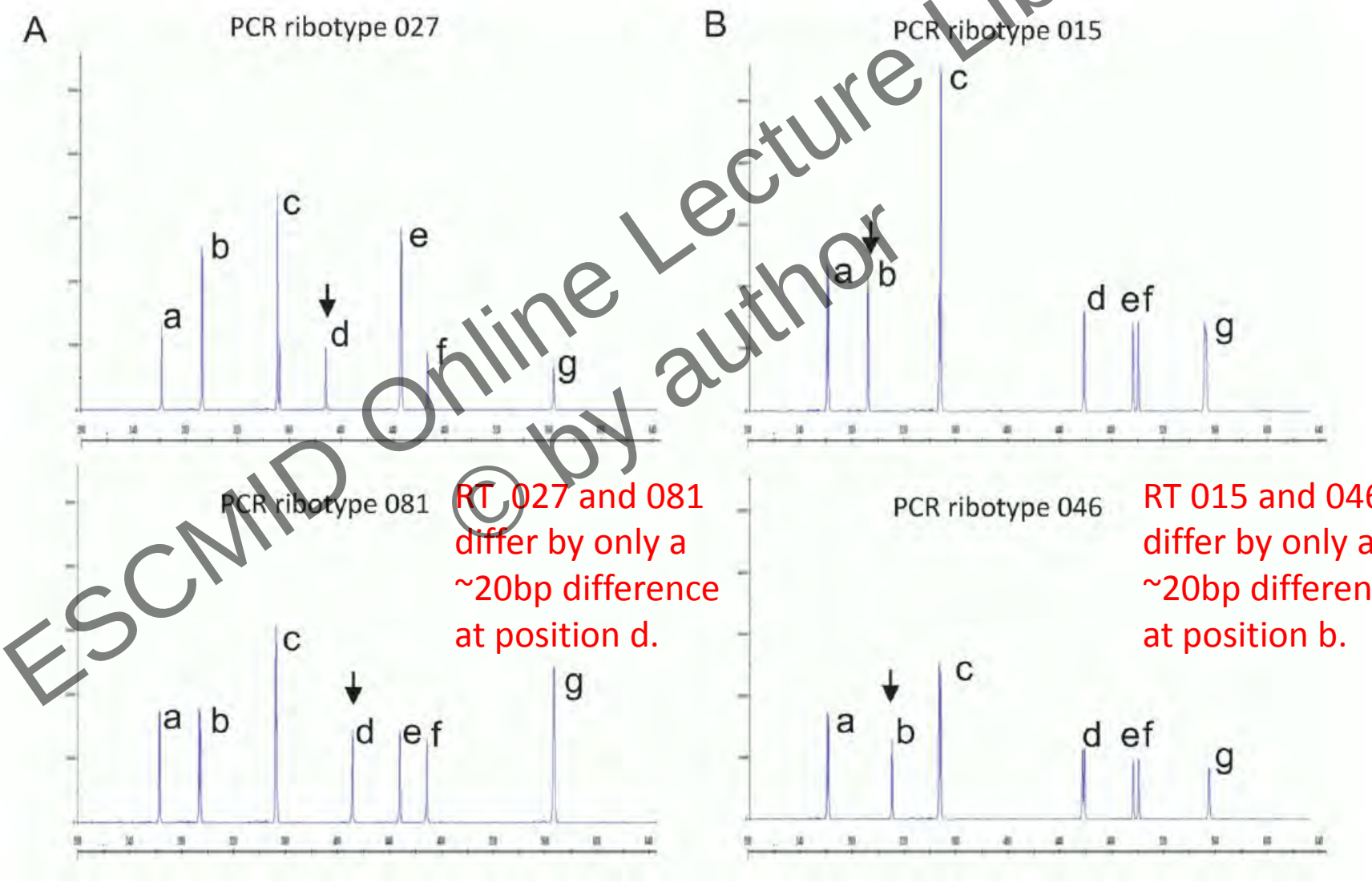
**Warren N. Fawley[1], C. W. Knetsch[3], Duncan R. MacCannell[2], Celine Harmanus[3], Tim Du[4], Michael R. Mulvey[4], Ashley Paulick[2], Lydia Anderson[2], E. J. Kuijper[3], Mark H. Wilcox[1,5]***

**1** Department of Microbiology, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom, **2** Centers for Disease Control and Prevention (CDC), Atlanta, United States of America, **3** Department of Medical Microbiology, Centre of Infectious Diseases, Leiden University Medical Centre, Leiden, Netherlands, **4** Public Health Agency of Canada (PHAC), Winnipeg, Canada, **5** Leeds Institute of Molecular Medicine, University of Leeds, Leeds, United Kingdom

* mark.wilcox@leedsth.nhs.uk mark.wilcox@nhs.net

# PCR-ribotypes with very similar profiles:

(a) ribotypes 027 & 081; (b) ribotypes 015 & 046.




RT 027 and 081 differ by only a ~20bp difference at position d.

RT 015 and 046 differ by only a ~20bp difference at position b.



http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4332677/pdf/pone.0118150.pdf

# PCR-ribotypes with very similar profiles:

(a) ribotypes 027 & 081; (b) ribotypes 015 & 046.

| | Fragment size (bp) | | | |
|---|---|---|---|---|
| Fragment | PCR ribotype 027 | PCR ribotype 081 | PCR ribotype 015 | PCR-ribotype 046 |
| a | 232.16 | 232.16 | 230.39 | 231.21 |
| b | 264.01 | 264.23 | **263.72** | **284.83** |
| c | 323.72 | 323.46 | 323.02 | 323.55 |
| d | **361.56** | **383.30** | 441.53 | 441.78 |
| e | 423.00 | 422.55 | 480.91 | 483.26 |
| f | 443.46 | 443.23 | 485.64 | 487.77 |
| g | 542.28 | 542.12 | 539.02 | 544.95 |



- RT 027 and 081 differ by only a ~20bp difference at position d.
- RT 015 and 046 differ by only a ~20bp difference at position b.
- Relative fragment size was the only parameter used to discriminate between ribotype profiles.
- Relative peak heights (relative fluorescent units, y-axis) within profiles lacked reproducibility for some ribotypes and therefore this parameter was not used.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4332677/pdf/pone.0118150.pdf

# Study conclusions

- A maximum SD of only ±3.8 bp was recorded in individual fragment sizes
- PCR ribotypes from 98.2% of anonymised strains were successfully discriminated across 4 ribotyping centres in Europe & N. America
- Consensus CE-ribotyping increases comparability of typing data between centres
- Consensus CE-ribotyping facilitates the rapid and accurate transfer of standardized typing data to support national and international *C. difficile* surveillance programs.

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RESEARCH ARTICLE

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1 Department of Microbiology, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom, 2 Centers for Disease Control and Prevention (CDC), Atlanta, United States of America, 3 Department of Medical Microbiology, Centre of Infectious Diseases, Leiden University Medical Centre, Leiden, Netherlands, 4 Public Health Agency of Canada (PHAC), Winnipeg, Canada, 5 Leeds Institute of Molecular Medicine, University of Leeds, Leeds, United Kingdom

* mark.wilcox@leedsth.nhs.uk



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**Data Availability Statement:** All relevant data are within the paper. Data on isolates in the panel have been made available online in a National Center for Biotechnology Information BioProject database (NCBI) (http://www.ncbi.nlm.nih.gov/bioproject/248340).

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## Abstract

PCR-ribotyping has been adopted in many laboratories as the method of choice for *C. difficile* typing and surveillance. However, issues with the conventional agarose gel-based technique, including inter-laboratory variation and interpretation of banding patterns have impeded progress. The method has recently been adapted to incorporate high-resolution capillary gel-based electrophoresis (CE-ribotyping), so improving discrimination, accuracy and reproducibility. However, reports to date have all represented single-centre studies and inter-laboratory variability has not been formally measured or assessed. Here, we achieved in a multi-centre setting a high level of reproducibility, accuracy and portability associated with a consensus CE-ribotyping protocol. Local databases were built at four participating laboratories using a distributed set of 70 known PCR-ribotypes. A panel of 50 isolates and 60 electronic profiles (blinded and randomized) were distributed to each testing centre for PCR-ribotype identification based on local databases generated using the standard set of 70 PCR-ribotypes, and the performance of the consensus protocol assessed. A maximum standard deviation of only ±3.8bp was recorded in individual fragment sizes, and PCR-ribotypes from 98.2% of anonymised strains were successfully discriminated across four ribotyping centres spanning Europe and North America (98.8% after analysing discrepancies). Consensus CE-ribotyping increases comparability of typing data between centres and thereby facilitates the rapid and accurate transfer of standardized typing data to support future national and international *C. difficile* surveillance programs.

## *Work package 3;* Establishing a European ribotyping nomenclature reference database for *Clostridium difficile* in close collaboration with ECDC (TESSy)

**Work package leader: dr.Val Hall, ARU, Cardiff, UK.**

- Objective 1. Build up and maintain a ribotyping nomenclature reference database for *Clostridium difficile*
- Objective 2. Provide free of charge service to MS reference laboratories for sharing *C. difficile* reference strains
- Objective 3. Provide a written document on SOPs and propose a guideline for the ribotyping of *Clostridium difficile isolates* in EU
- Objective 4. Provide External Quality Assesment (EQA) for national reference laboratories in the MS for ribotyping and assessment of antimicrobial resistance of *C. difficile* strains (yearly or 6-monthly


The Leeds Teaching Hospitals NHS
NHS Trust



4th CDRN EQA RESULTS (distributed March 2010)

| Strain | Ribotype identity | Lab 1 | Lab 2 | Lab 3 | Lab 4 | Lab 5 | Lab 6 | Lab 7 | Lab 8 | Lab 9 | Lab 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| EQA1 | 078 | 078 | 078 | 078 | 078 | 078 | 078 | 078 | 078 | 078 | 078 |
| EQA2 | 005 | 005 | 005 | 005 | 005 | 005 | 005 | 005 | 005 | 005 | 005 |
| EQA3 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 | 106 |
| EQA4 | 027 | 027 | 027 | 027 | 027 | 027 | 027 | 027 | 027 | 027 | 027 |
| EQA5 | 081 | 081 | 081 | 035 | 081 | 081 | 081 | NI | 081 | 081 | 081 |
| EQA6 | 020 | 020 | 014/20 | 020 | 020 | 014 | 106 | 014 | 020 | 020 | 020 |
| EQA7 | 017 | 017 | 017 | 017 | 017 | 017 | 017 | 017 | 017 | 017 | NI |
| EQA8 | 015 | 015 | 015 | 015 | 015 | 015 | 015 | 015 | 015 | 015 | 015 |
| EQA9 | 001 | 001 | 001 | 001 | 001 | 001 | 001 | 001 | 001 | 001 | 001 |
| EQA10 | 174 | 174 | 174 | 174 | 174 | 174 | 174 | 174 | 174 | 174 | NI |
| % | - | 100 | 100 | 90 | 100 | 90 | 90 | 80 | 100 | 100 | 90 |

# The way forward

- ECDC supported pan-Europe collaboration to provide:
  - Web-based reference ribotyping service
  - Single nomencalture
  - Based on data submission / sharing
  - Limited distribution of reference strains

Typing vs fingerprinting

Real world examples

Compare geno'typing' methods

Whole genome sequencing

Problems and solutions

Discussion