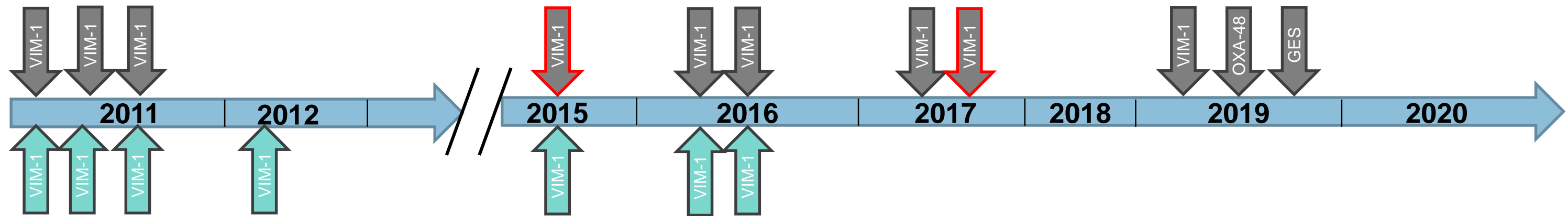


# Isolation Procedure for Carbapenemase-producing *E. coli* from Caeca Samples under Review

15th EURL-AR Workshop 2021

**Natalie Pauly**

# Carbapenemase-producing Enterobacteriaceae (CPE) in the German livestock



- ❖ Since 2011: Detections of the carbapenemase VIM-1 in *E. coli* and *S. Infantis* in German livestock (pigs)
- ❖ In 2017: CPE isolation method failed for one isolate, which was recovered within the monitoring of ESBL/AmpC producing *E. coli*
- ❖ Up to 2019: High similarity of the  $bla_{VIM-1}$ -carrying plasmids, also across species!
- ❖ In 2019: Increased variance (VIM-1, OXA-48, and GES-producing *E. coli*)

# IMPART



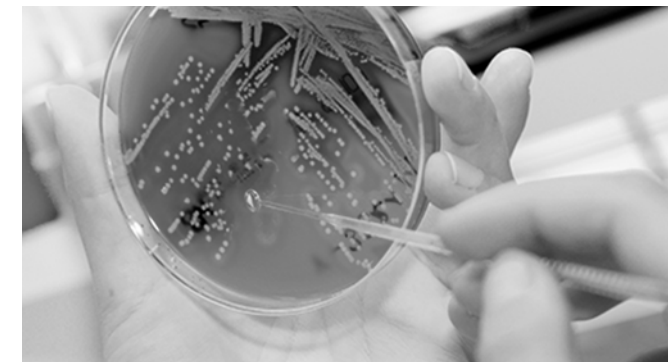
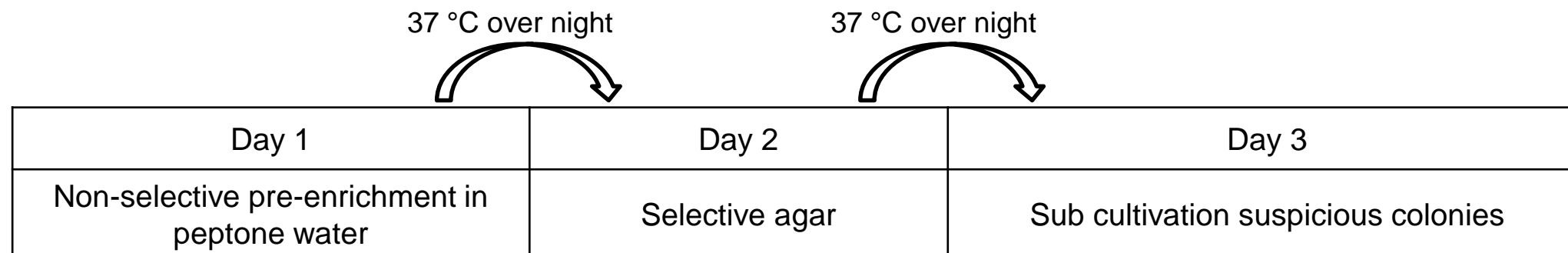
## Improving phenotypic testing of AMR by development of sensitive screening assays for emerging resistance and setting missing ECOFFs

WP 2: Selective isolation, detection and characterization of carbapenemase-producing Enterobacteriaceae

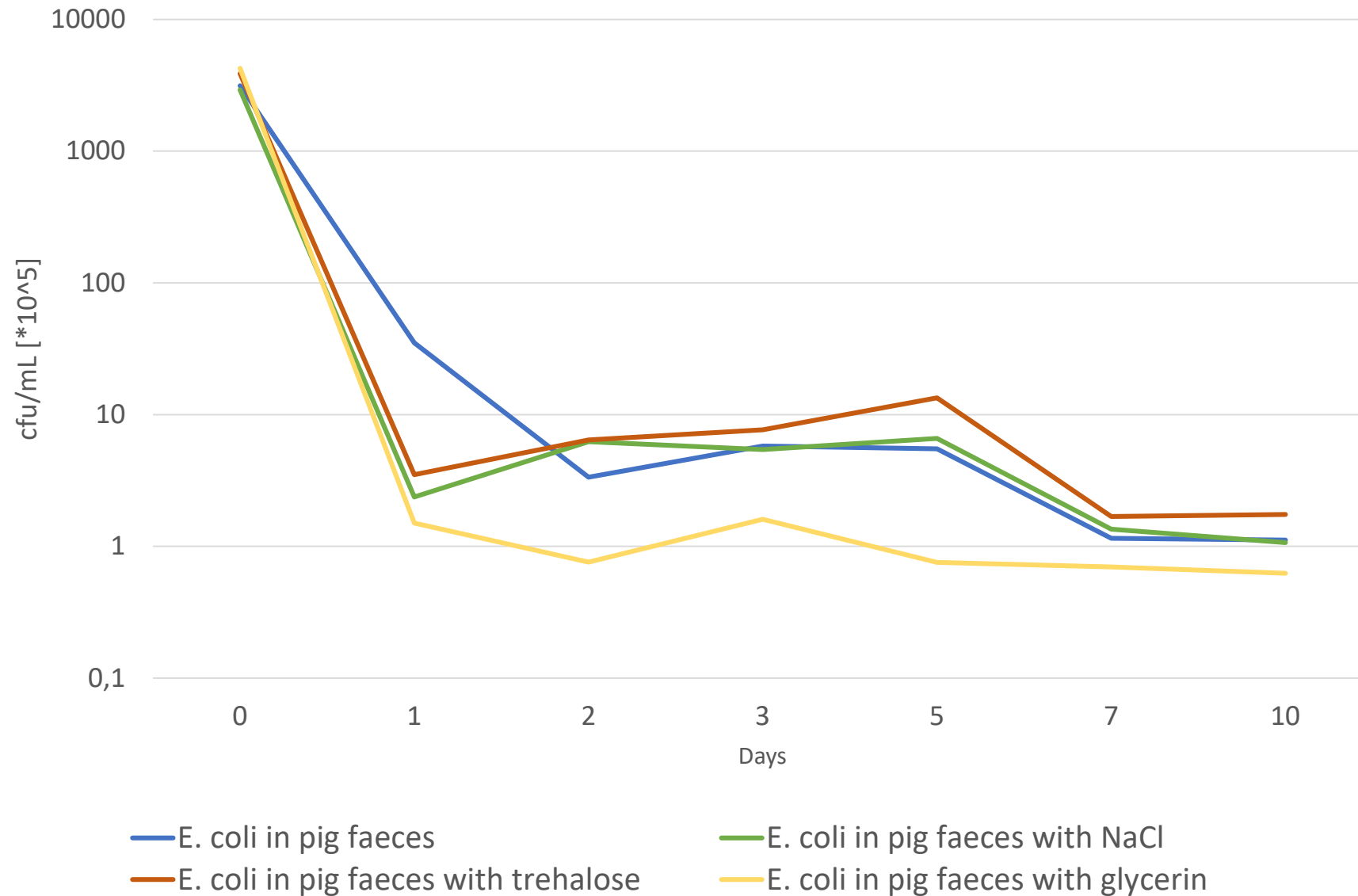
With the beginning of 2018, the European joint project IMPART was launched as a part of the One Health EJP Programme.

- ❖ The optimization of the commonly used isolation method for CPE by variation of microbiological parameters
- ❖ The validation of the modified method

# EURL-protocol: Isolation method for CPE



# Survival rate of CP *E. coli* in fecal samples



“This should be initiated as soon as possible after receipt in the laboratory, preferably within **24 hours**”

DTU Food  
National Food Institute



## LABORATORY PROTOCOL

Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from fresh meat

February 2018  
Version 6

Version 6 reviewed and updated by: Rene S. Hendriksen and Valeria Bortolaia

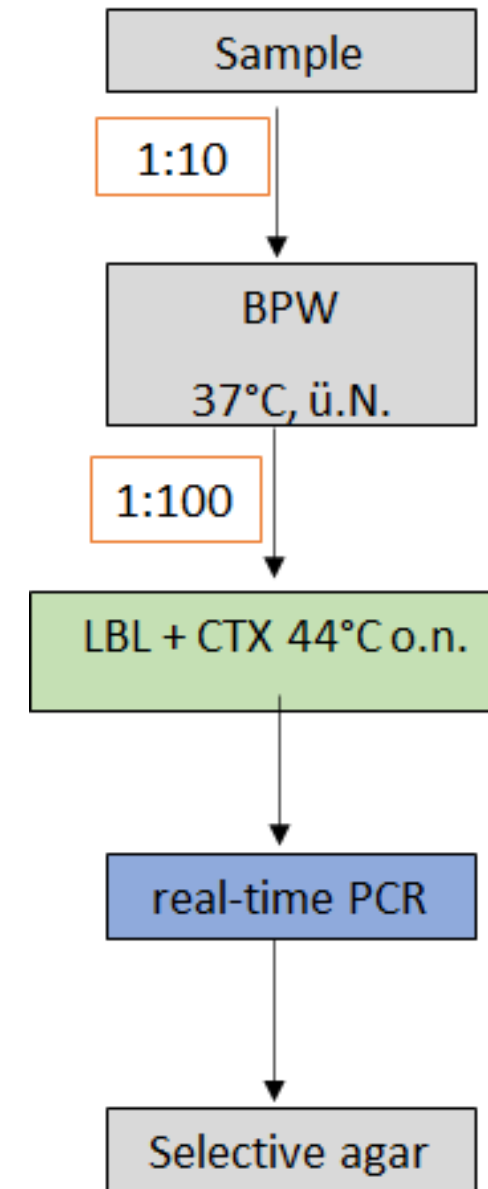
Authors of the document: Henrik Hasman, Yvonne Agersø, Rene Hendriksen, Lina M. Cavaco (DTU Food) and Beatriz Guerra-Roman (external expert)

# The optimization by variation of microbiological parameters



## Characterization of VIM-1-Producing *E. coli* Isolated From a German Fattening Pig Farm by an Improved Isolation Procedure

A. Irrgang<sup>1\*</sup>, B.-A. Tenhagen<sup>1</sup>, N. Pauly<sup>1</sup>, S. Schmogger<sup>1</sup>, Annemarie Kaesbohrer<sup>1,2</sup> and J. A. Hammerl<sup>1</sup>



BPW: Buffered peptone water  
LB: Lysogeny broth  
McC: Mc Conkey agar

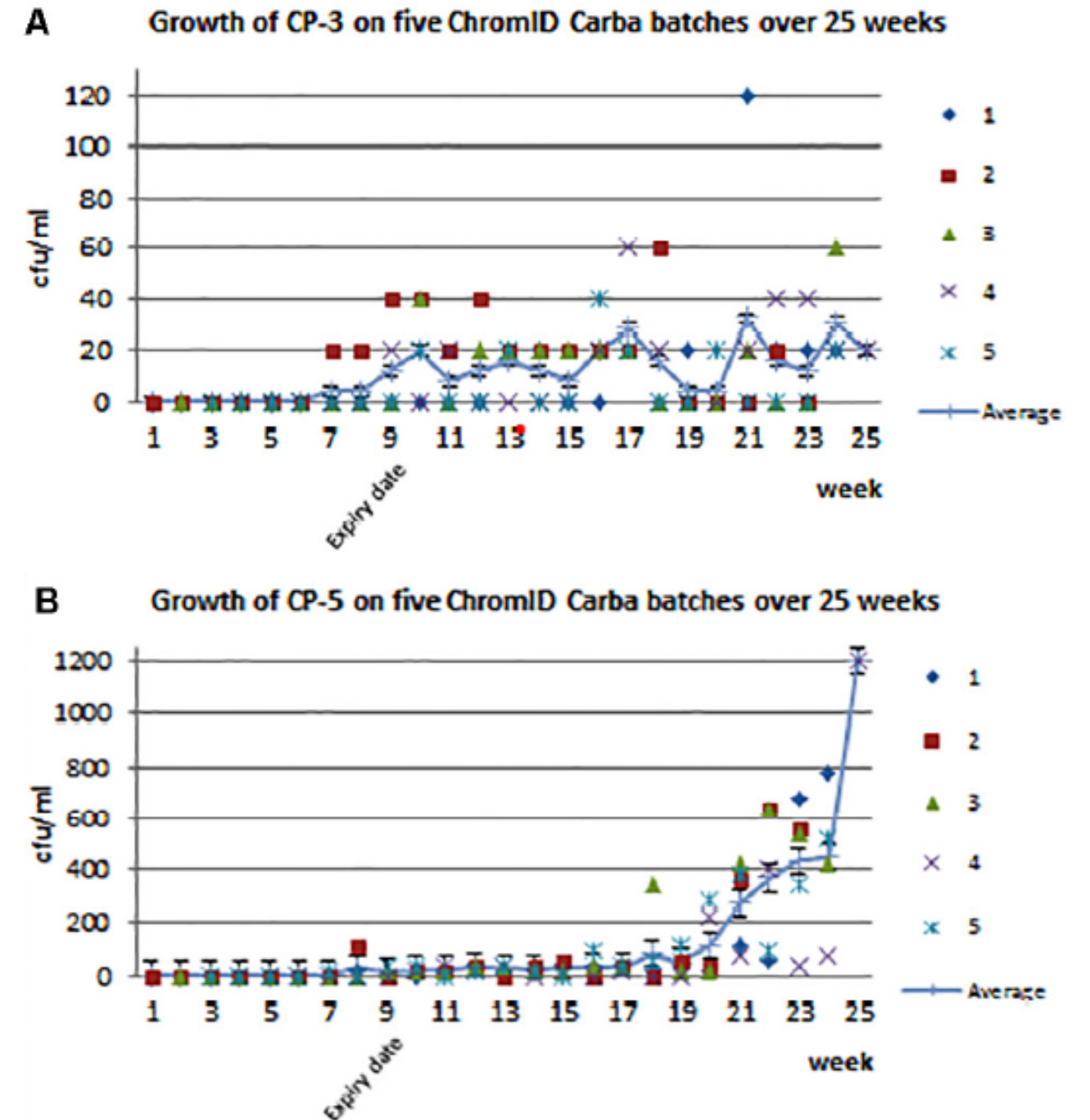
CTX: Cefotaxime  
MEM: Meropenem

# The optimization by variation of microbiological parameters

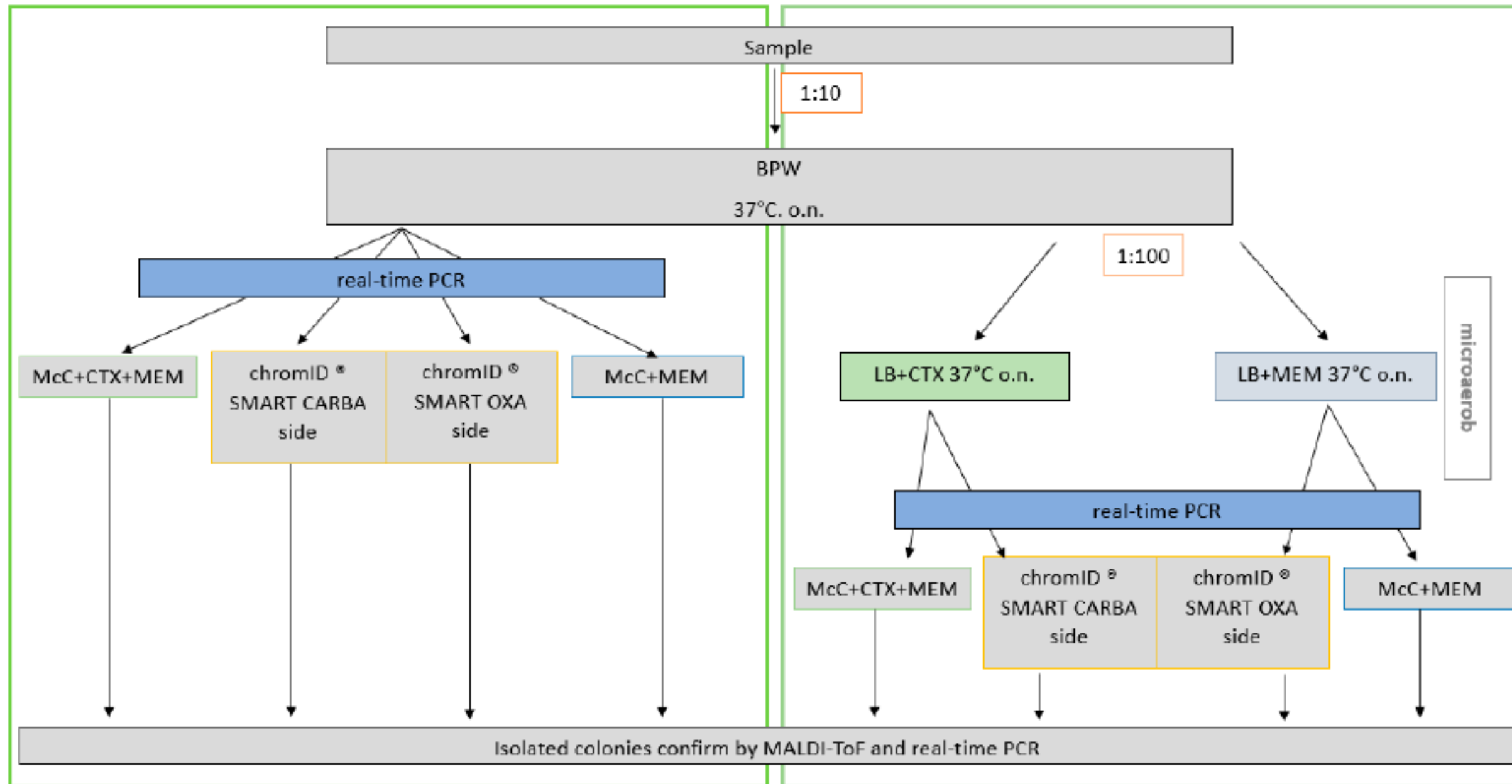


## ChromID<sup>®</sup> CARBA Agar Fails to Detect Carbapenem-Resistant *Enterobacteriaceae* With Slightly Reduced Susceptibility to Carbapenems

Natalie Pauly<sup>1\*</sup>, Jens A. Hammer<sup>1</sup>, Mirjam Grobber<sup>1</sup>, Bernd-Alois Tenhagen<sup>1</sup>, Annemarie Käsbohrer<sup>1,2</sup>, Sandra Bisenius<sup>3</sup>, Jannika Fuchs<sup>4</sup>, Sabine Horlacher<sup>5</sup>, Holger Lingstädt<sup>6</sup>, Ute Mauermann<sup>7</sup>, Silke Mitro<sup>8</sup>, Margit Müller<sup>9</sup>, Stefan Rohrmann<sup>10</sup>, Arthur P. Schiffmann<sup>11</sup>, Birgit Stöhrenberg<sup>12</sup>, Pia Zimmermann<sup>13</sup>, Stefan Schwarz<sup>14</sup>, Diana Meemken<sup>15</sup> and Alexandra Irrgang<sup>1\*</sup>



# The optimization by variation of microbiological parameters



BPW: Buffered peptone water  
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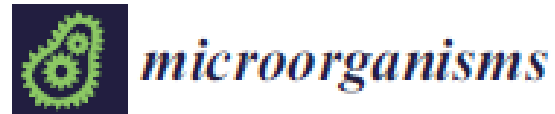
CTX: Cefotaxime  
 MEM: Meropenem

Method A

Method B









# The optimization by variation of microbiological parameters



Article

## Isolation Procedure for CP *E. coli* from Caeca Samples under Review towards an Increased Sensitivity

Natalie Pauly<sup>1</sup>, Yvonne Klar<sup>1</sup>, Tanja Skladnikiewicz-Ziemer<sup>1</sup>, Katharina Juraschek<sup>1</sup>, Mirjam Grobbel<sup>1</sup> , Jens André Hammerl<sup>1</sup> , Lukas Hemmers<sup>2</sup>, Annemarie Käsbohrer<sup>1,3</sup> , Stefan Schwarz<sup>4,5</sup> , Diana Meemken<sup>6</sup> , Bernd-Alois Tenhagen<sup>1</sup> and Alexandra Irrgang<sup>1,\*</sup> 

	Method A + ChromID <sup>®</sup> SMART CARBA agars	Method A + <i>in-house</i> agars	Method B + <i>in-house</i> agars	Method B + ChromID <sup>®</sup> SMART CARBA agars
Sensitivity	75 (57.8–87.9)	100 (90.2–100)	86.1 (70.5–95.3)	66.7 (49–81.4)
Specificity	100 (81.5–100)	100 (81.5–100)	100 (81.5–100)	100 (81.5–100)
False discovery rate	0 (0–12.8)	0 (0–9.7)	0 (0–11.2)	0 (0–14.2)
False omission rate	33.3 (16.5–54)	0 (0–18.5)	21.7 (7.5–43.7)	40 (22.7–59.4)
Accuracy	83.3 (70.7–92.1)	100 (93.4–100)	90.7 (79.7–96.9)	77.8 (64.4–88)

## It could be summarized that...

- ❖ Potential additives did not have a positive effect on the survival rate of CPE in faeces samples
  - ❖ Processing the samples in a timely manner, preferably within 24 hours
  - ❖ The choice of selective agar is crucial
- 
- Therefore, we recommend the EURL-method with a selective medium corresponding to the searched target bacterium
  - A second enrichment under microaerophilic conditions reduced the accompanying microbiota, but the sensitivity of the whole procedure was decreased from 100% to 86.1%.



# Thank you for your attention

Natalie Pauly

Bundesinstitut für Risikobewertung

Max-Dohrn-Str. 8-10 • 10589 Berlin

Tel. 030 - 184 12 - 0 • Fax 030 - 184 12 – 24337

natalie\_pauly@hotmail.com / alexandra.irrgang@bfr.bund.de •

www.bfr.bund.de