General guidelines for laboratory detection of carbapenemase-producing *Enterobacterales* (CPE) in Belgium

NRC Antibiotic-resistant Gram-negative bacilli

Carbapenemase types in *Enterobacterales*, *Pseudomonas* spp. and *Acinetobacter* spp. vs

Ambler molecular class	Carbapenemase family	*Enterobacterales* (CPE)	*Pseudomonas* spp.	*Acinetobacter* spp.
A (serine)	**KPC**	**+++**	-	-
	GES	(+)	+	(+)
	IMI, NMC, SME, SFC…	+	-	-
B (zinc MBL)	**VIM,**	**+++**	+++	+
	NDM	**+++**	+	++
	IMP	+	+	+
	AIM, DIM, GIM, SIM, SPM…	-	(+)	(+)
D (serine)	**OXA-48-like**	**+++**	-	-
	OXA-427	+	-	-
	OXA-198	(+)	+	-
	OXA-23-like	(+)	-	+++
	OXA-24-like	(+)	-	++
	OXA-58-like	(+)	-	++

Selection criteria for suspicion of CPE

- **Species identification confirmed belonging to *Enterobacterales***
- **Decreased susceptibility to at least one carbapenem using screening breakpoint** (not clinical S breakpoint)**:**
  - MIC method: **ertapenem OR meropenem > 0.125 mg/L** (Sensitivity of 95%)
    - NB: automated systems may have insufficient sensitivity if lowest concentration does not reach 0.125 mg/l
  - Disk diffusion method:
    - **Ertapenem (10 µg) < 25 mm** (Sensitivity of 98-99%) OR
    - **Meropenem (10 µg) <28 mm** (Sensitivity of 95%)

If carba non-S confirmed → Perform first-line screening tests

First-line screening tests to be performed by local laboratories

At least one test positive of the following :

- **High-level resistance to ceftazidime/avibactam CAZ/AVI :**
  - Disk diffusion: CAZ/AVI (10/4 µg) <10 mm OR
  - MIC method: CAZ/AVI >16 mg/L

OR

- **High-level resistance to temocillin TMO :**
  - Disk diffusion: TMO (30 µg) <12 mm OR
  - MIC method: TMO >64 mg/L

OR

- **Carbapenemase hydrolysis-based test (HBT) : one of the following**
  - **Colorimetric tests:**
    - Rapid CARB Screen Kit, Rapid CARB Blue Kit (ROSCO)
    - RAPIDEC (BioMérieux)
    - Beta-CARBA test (BioRad)
  - **Mass spectrometry tests:**
    - MALDITOF MBT Star Carba (Bruker)
  - **Carbapenem inactivation methods (CIM)**
    - Modified CIM : mCIM, zCIM…

Second-line confirmatory tests (optional)

1) Immunochromatographic tests (ICT):

- Monoplex ICT: OXA-48 → 60% of all CPE in Belgium
- Multiplex ICT: OXA-48, KPC, NDM, VIM, IMP

2) Inhibitor-based combination (IBT) disk tests or gradient MIC tests:

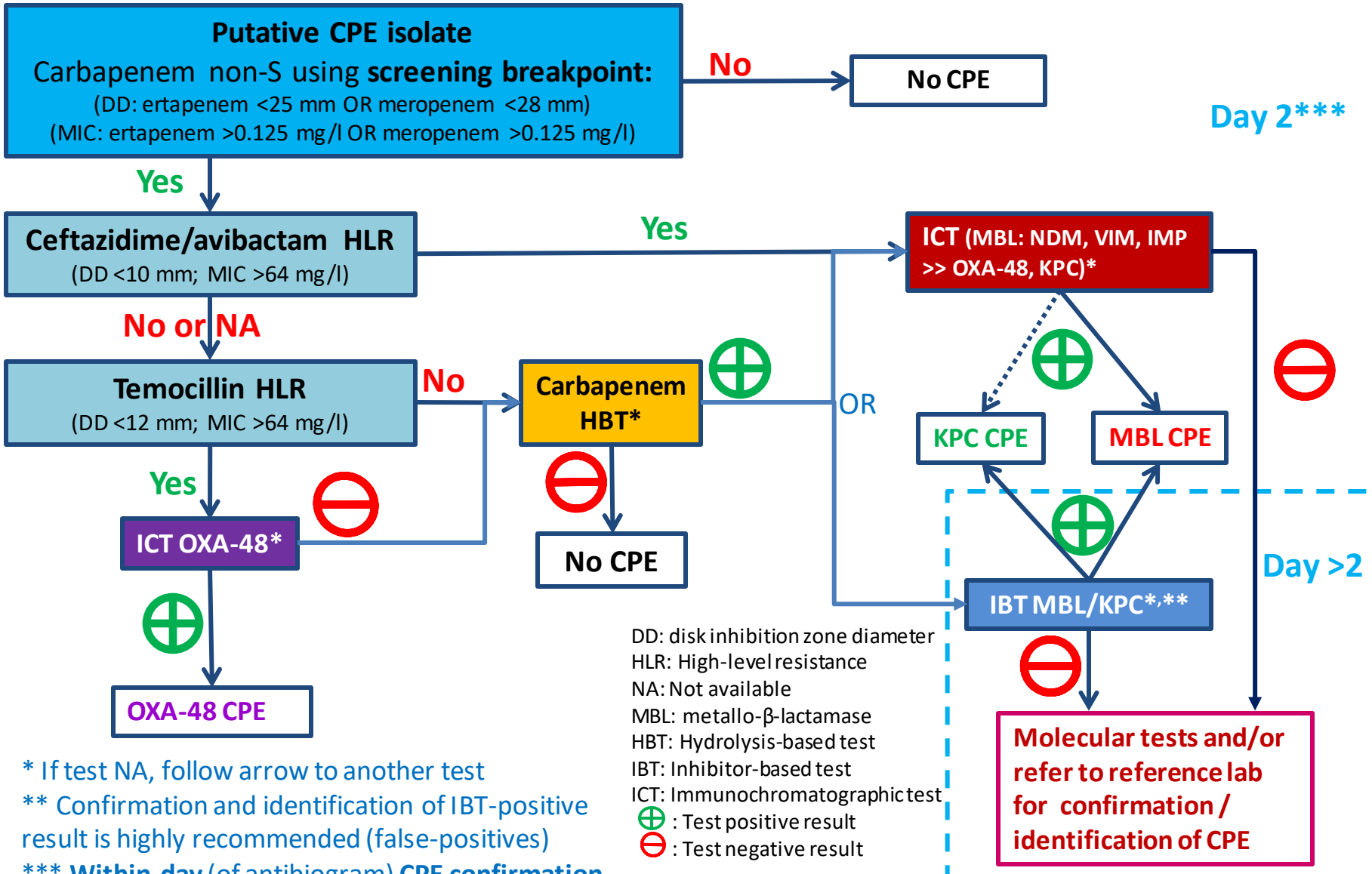
- Class B carbapenemase (MBL: VIM/NDM/IMP): Mero +/- DPA (dipicolinic acid) or Imi +/- EDTA
- Class A carbapenemase (KPC): Mero +/- BA (boronic acid) and mero +/- cloxacillin

3) Molecular tests (PCR assays, DNA microarray, isothermal amplification, sequencing…): identification of carbapenemase family and/or enzyme variant

General criteria to rejection of request or delay of analysis for confirmation of CPE by the NRC

- Sample received without reference ID, minimal sample **information** (sample nature / date), isolate species identification, copy of antibiogram results,…
- **Damaged** samples (crushed plates, broken glass tubes,….)
- **Contaminated** culture
- Isolate received at the NRC identified as a **different species** from the one notified on the request form
- Isolate belonging to genera/species **other than *Enterobacterales***
- Isolate with **no decreased susceptibility** to any carbapenem (erta, mero) according to screening criteria
- **No first-line nor second-line tests** being performed by local laboratories
- Repeated isolates of **same species from the same patient** with similar resistance patterns

Algorithm for detection of CPE on isolates



DD: disk inhibition zone diameter
HLR: High-level resistance
NA: Not available
MBL: metallo-β-lactamase
HBT: Hydrolysis-based test
IBT: Inhibitor-based test
ICT: Immunochromatographic test
⊕ : Test positive result
⊖ : Test negative result

* If test NA, follow arrow to another test
** Confirmation and identification of IBT-positive result is highly recommended (false-positives)
*** **Within-day** (of antibiogram) **CPE confirmation**