Detection of OXA-48-like, KPC and NDM carbapenemases directly from blood culture bottles using an immunochromatographic assay

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Working

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ABSTRACT

Bloodstream infections caused by carbapenemase-producing Enterobacteriaceae (CPE) are associated with treatment failure and increased mortality. Detection of CPE from blood cultures (BC) by standard methods takes 16-72 hours, which can delay the initiation of appropriate antimicrobial therapy and compromise patient outcome. We developed and evaluated a new method for the rapid detection of carbapenemases directly from positive BC using a new multiplex immunochromatographic test (ICT), the RESIST-3 O.K.N. ICT® (Coris BioConcept, Gembloux, Belgium). It was originally developed for the detection of OXA-48-like, KPC and NDM carbapenemases from bacteria grown on solid media.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL STATUS

Working

GUIDELINES

Follow guidelines for good laboratory practice (GLP)

MATERIALS

NAME	CATALOG #
PBS	Contributed by users
double distilled water (ddH2O)	Contributed by users
10% SDS solution	Contributed by users
0,5 M zinc sulfate (ZnSO4)	Contributed by users
1.5 ml reaction tube	by Eppendorf
RESIST-3 O.K.N. immunochromatographic assay	by Coris bioConcept

BEFORE STARTING

Prepare

10% SDS (can be stored for several weeks, but make sure that it has not crystallized)

0.5 M $ZnSO_4$ (can be stored for months)

Other reagents required:

double distilled water ddH_20

RESIST-3 O.K.N. ICT (Coris BioConcept, Gembloux, Belgium)

1.5 ml reaction tubes (e.g. Eppendorf)

1 Draw 1-3 ml of blood culture fluid from blood culture bottles that have been flagged positive by the blood culture automate (e.g. BD Bactex FX) and that contain Gram-negative bacilli on Gram-stain.
Add 1 ml of blood culture fluid in a 1.5 ml reaction tube (e.g. Eppendorf).

Optional step - incubation with ZnSO4 for improved performance with NDM-producing Enterobacteriaceae (advanced protocol)

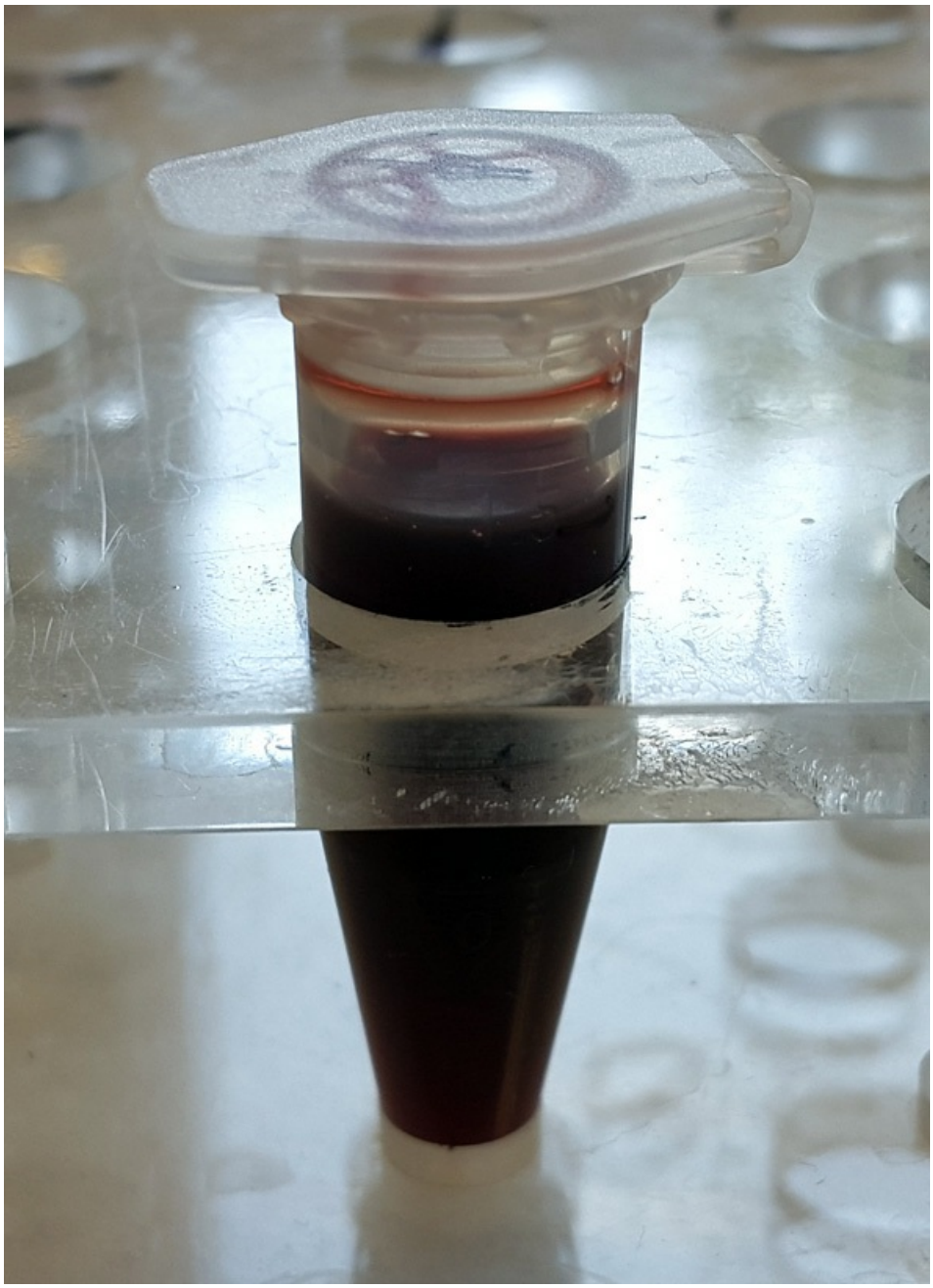
2 Add 10 µl $ZnSO_4$ solution and incubate in a heating block for 15 min. at 37°C, shaking at 300/min.

(incubation at room temperature without shaking might be possible, but has not been extens

ively tested)

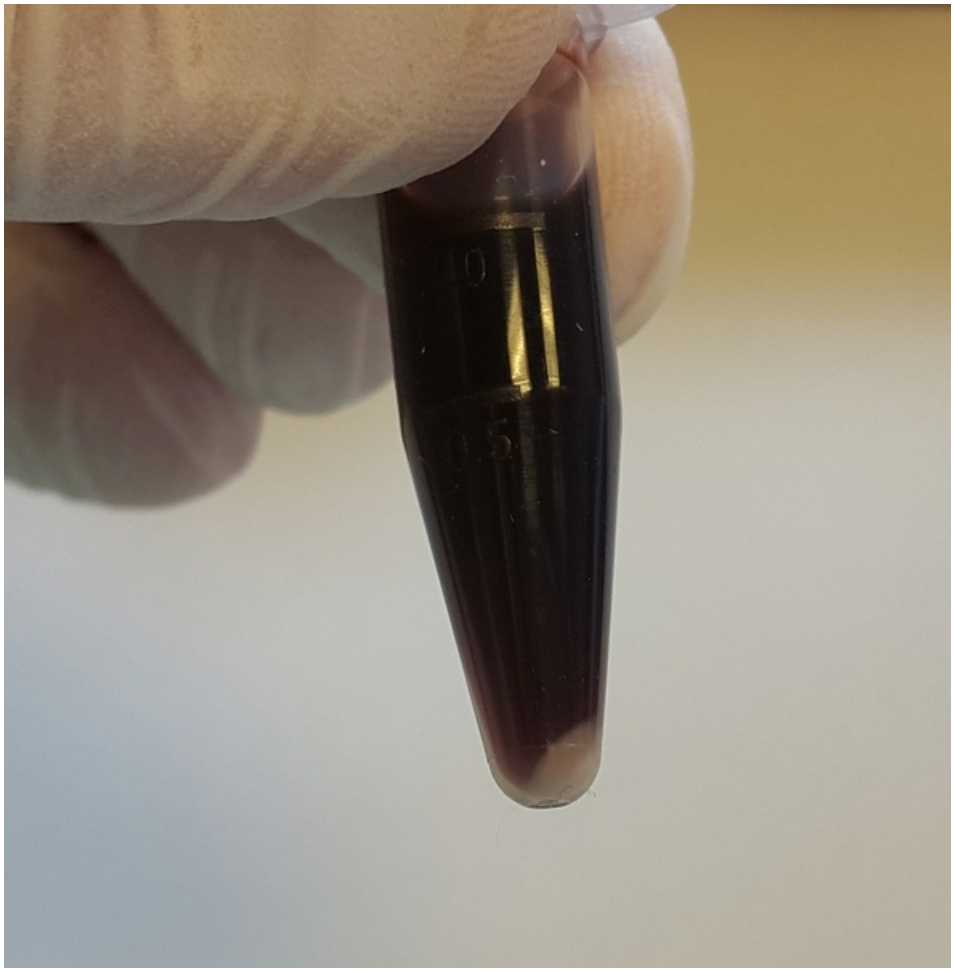
Hemolysis of red blood cells

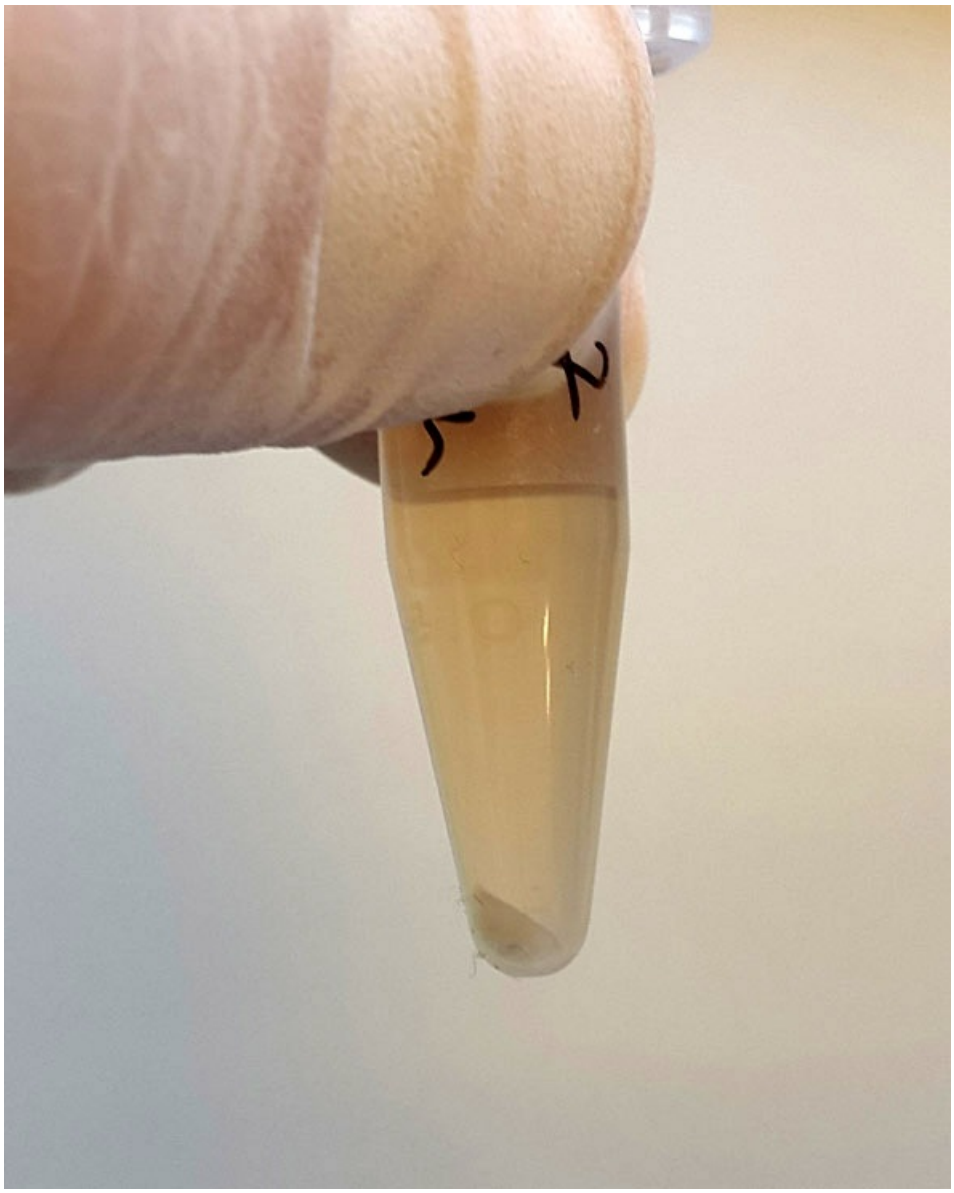
3 Add 200 µl of 10% SDS to the reaction tube, invert and vortex.
Incubate for 5 min. at room temperature.
Centrifuge for 1 min @ 13 000 g in a conventional microcentrifuge



Washing of bacteria

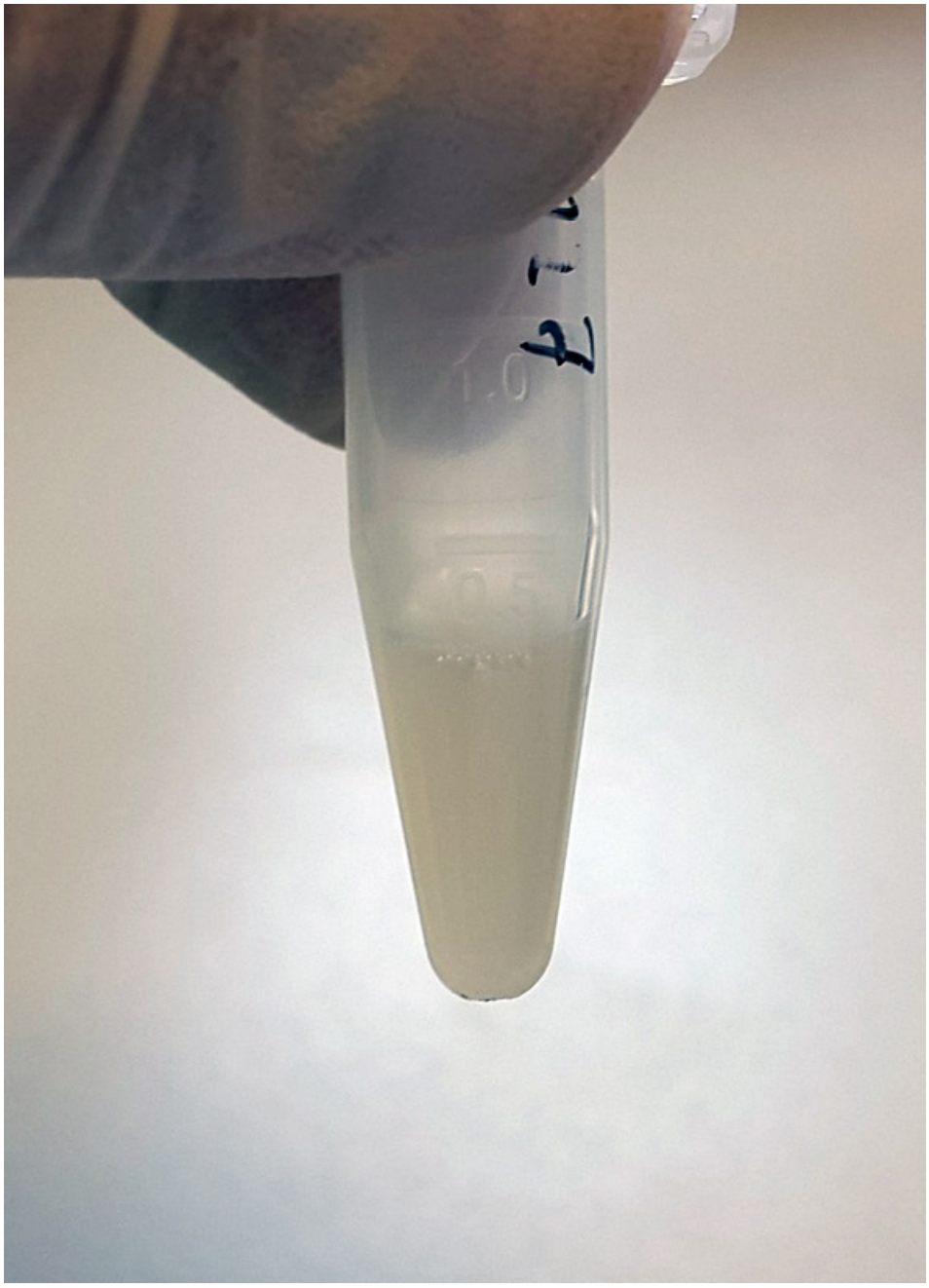
4 After centrifugation, a white pellet should be visible at the bottom of the reaction tube (see picture).
This pellet should not be smaller than what is shown in the picture. If the pellet is smaller, use a larger volume of blood culture fluid.
Gently remove the supernatant with a pipette, taking care NOT to disturb the pellet.
Add 1 ml of ddH_20, vortex or resuspend with a pipette. Make sure that a homogenous suspension is achieved!
Centrifuge again for 1 min @ 13000 RPM.
Remove the supernatant
If the pellet is white/grey without many erythrocytes, go on to the next step.
If the pellet is still red, repeat the washing step with ddH
2
0 or PBS





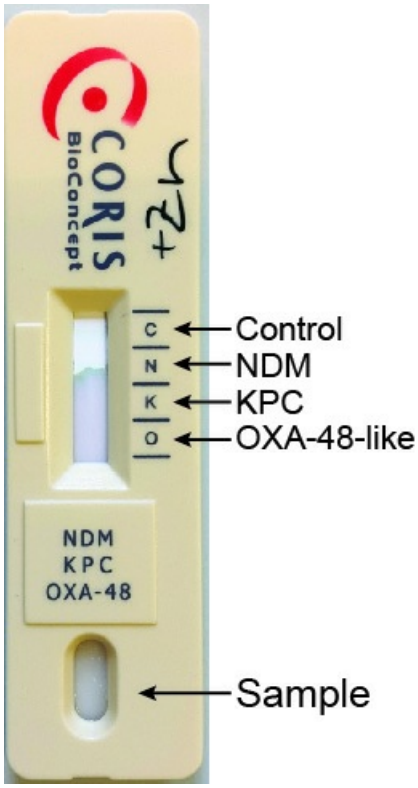
Lysis of bacteria

5 Remove the supernatant gently.
Snap the bottom of the reaction tube with a finger and/or vortex, so that the pellet does not adhere so firmly to the walls of the tube.
Add 10 drops of LY-A solution (comes with the RESIST-3 O.K.N.) and vortex. Make sure that a homogenous suspension is achieved (e.g. by vortexing or pipetting up and down).



Loading of the immunochromatographic assay

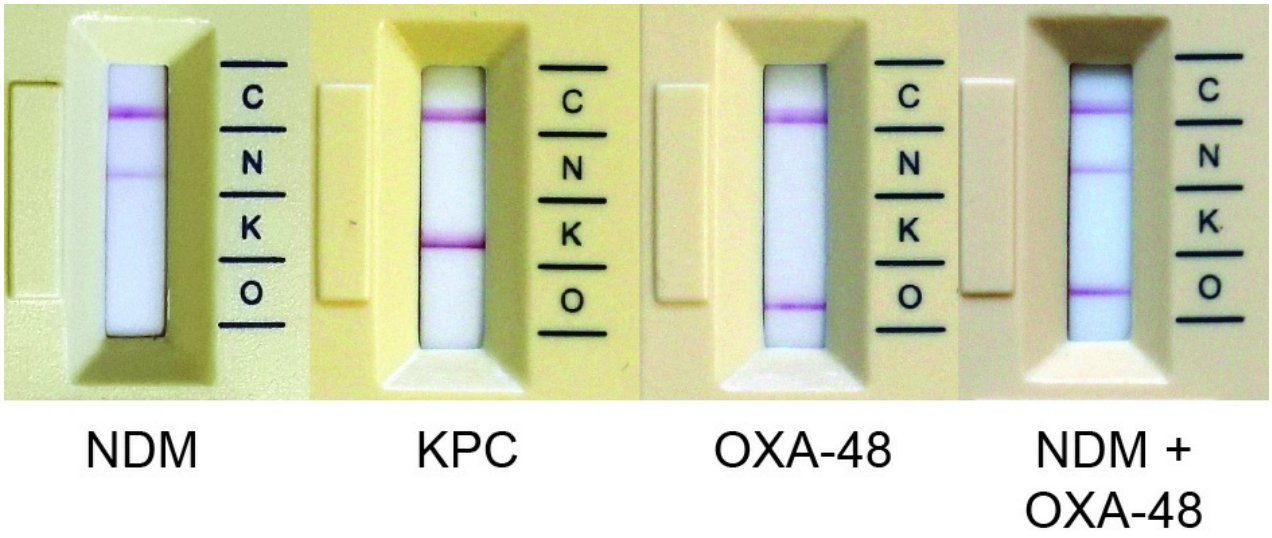
6 Add 90 µl of the suspension in the sample field of the cassette test.
Incubate up to 15 min at room temperature.



Reading of the immunochromatographic assay

7 The test is valid if a red line appears at the position marked with 'C' (=control line).
OXA-48 isolates show an additional band at the position marked with 'O', KPC producing isolates at the position marked with 'K', NDM isolates at the position marked with 'N'.
OXA-48-like and KPC producing isolates usually show a positive reaction after 30s to 2 min, while NDM-producing isolates take longer (usually 2-7 min, sometimes up to 15 min). NDM bands are fainter than bands of OXA-48 or KPC.
Any band appearing on the ICT within the maximum reading time of 15 min. should be regarded as positive!

Tipps:
Some isolates have more than one carbapenemase. If a band has appeared at the position of KPC or OXA-48 after 1-2 min and you want to exclude the additional presence of an NDM carbapenemase, read the test a second time at 15 min.



Tipps & Troubleshooting

8
- Test the ICT with several previously characterized isolates producing the carbapenemases OXA-48, KPC and NDM to get familiar with the test procedure.
- If the pellet (or a large part thereof) is lost during washing/centrifugation or if the pellet is too small from the beginning, repeat steps 1-4 to obtain a sufficiently large pellet before continuing with the protocol.
  If the pellet (corresponding to the bacterial inoculum) is too small, false-negative results can occur, especially for NDM.
- The short protocol (without step 2) works well for all OXA-48 and KPC and most NDM-producing Enterobacteriaceae. Some NDM-producing isolates give rise to very faint bands, which can be improved by the additional 15 min (or longer) incubation with $ZnSO_4$ (advanced protocol). Zinc is a co-factor for metallo-beta-lactamases, such as NDM.

Results

9 The assay has been assessed in a proof of principle study using spiked blood cultures.
For this purpose, 170 molecularly characterized Enterobacteriaceae isolates have been tested; of these, 126 produced a carbapenemase of the types NDM, OXA-48-like or KPC.
Sensitivity and specificity were 100%.
However, it has not been validated with clinical samples. Further studies are necessary to assess the performance of this test in routine diagnostics.