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Evaluation of the BYG Carba Test, a New Electrochemical Assay for Rapid Laboratory Detection of Carbapenemase-Producing *Enterobacteriaceae*

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Accurate detection of carbapenemase-producing *Enterobacteriaceae* (CPE) constitutes a major laboratory diagnostic challenge. We evaluated an electrochemical technique (the BYG Carba test) which allows detection of CPE in less than 35 min. The BYG Carba test was first validated in triplicate against 57 collection isolates with previously characterized β-lactam resistance mechanisms (OXA-48, *n* = 12; KPC, *n* = 8; NDM, *n* = 8; VIM, *n* = 8; IMP, *n* = 3; GIM, *n* = 1; GES-6, *n* = 1; no carbapenemase, *n* = 16) and against a panel of 10 isolates obtained from the United Kingdom National External Quality Assessment Service (NEQAS). The test was then evaluated prospectively against 324 isolates referred to the national reference center for suspicion of CPE. The BYG Carba test results were compared with those obtained with the Carba NP test using multiplex PCR sequencing as the gold standard. Of the 57 collection and the 10 NEQAS isolates, all but one GES-6-producing isolate were correctly identified by the Carba BYG test. Among the 324 consecutive *Enterobacteriaceae* isolates tested prospectively, 146 were confirmed as noncarbapenemase producers by PCR while 178 harbored a carbapenemase gene (OXA-48, *n* = 117; KPC, *n* = 25; NDM, *n* = 23; and VIM, *n* = 13). Prospectively, in comparison with PCR results, the BYG Carba test displayed 95% sensitivity and 100% specificity versus 89% and 100%, respectively, for the Carba NP test. The BYG Carba test is a novel, rapid, and efficient assay based on an electro-active polymer biosensing technology discriminating between CPE and non-CPE. The precise electrochemical signal (electrochemical impedance variations) allows the establishment of real-time objective measurement and interpretation criteria which should facilitate the accreditation process of this technology.

The worldwide emergence and dissemination of carbapenemase-producing Gram-negative rods, in particular, carbapenemase-producing *Enterobacteriaceae* (CPE) that are resistant to carbapenems, is a major public health concern. Rapid detection and confirmation of CPE are essential for appropriate choice of antimicrobial therapy as well as for the implementation and/or maintenance of appropriate infection control measures (1). The transmissible carbapenemases are divided into three different classes, class A (serine carbapenemases, such as KPC), class B (metallo-β-lactamases [MBLs], such as VIM, IMP, and NDM), and class D (OXA carbapenemases, such as OXA-48) (1–3).

Various phenotypic screening and confirmatory tests for the detection of carbapenemases have been proposed, including inhibition tests of carbapenemase activity, for example, combined-disk tests using specific inhibitors such as EDTA and boronic acid, the modified Hodge test (MHT) (4), the carbapenem inactivation method (5), and detection of carbapenem hydrolysis by the Carba NP test (6) or by other closely related tests (7, 8). However, although allowing, in some formats, the differentiation between class A and B carbapenemases (9), these tests cannot specify types within each class of carbapenemases, (e.g., IMP, VIM, NDM, SIM, and GIM in class B), and they also are unable to confirm in a single test the occurrence of class D OXA-48 carbapenemase (10). To confirm the presence of OXA-48, Tsakris and colleagues (11) also recently proposed a confirmatory disk test (96.3% sensitivity and 97.7% specificity) based on the use of an imipenem disk, EDTA, and a combination of EDTA and phenyl boronic acid.

Molecular detection methods such as PCR and sequencing of carbapenemase genes are nevertheless more reliable for confirmation of carbapenemases (10), but they are only rarely implemented in most routine clinical microbiology laboratories because of their high cost and the skill level and the special equipment required.

However, rapid non-molecular-based confirmatory testing of the presence of a carbapenemase can be sufficient to implement clinical management strategies and infection control measures to limit the spread and cross-transmission of these organisms.

Various confirmatory tests rely on direct monitoring of β-lactam hydrolysis. For example, a spectrophotometric method (12) is reported as a very specific and sensitive gold standard but requires a spectrophotometer and is applied only in specialized laboratories.

In 2011, two different groups demonstrated that matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) was able to detect by-products resulting from the hydrolysis of a carbapenem in the presence of a bacterial

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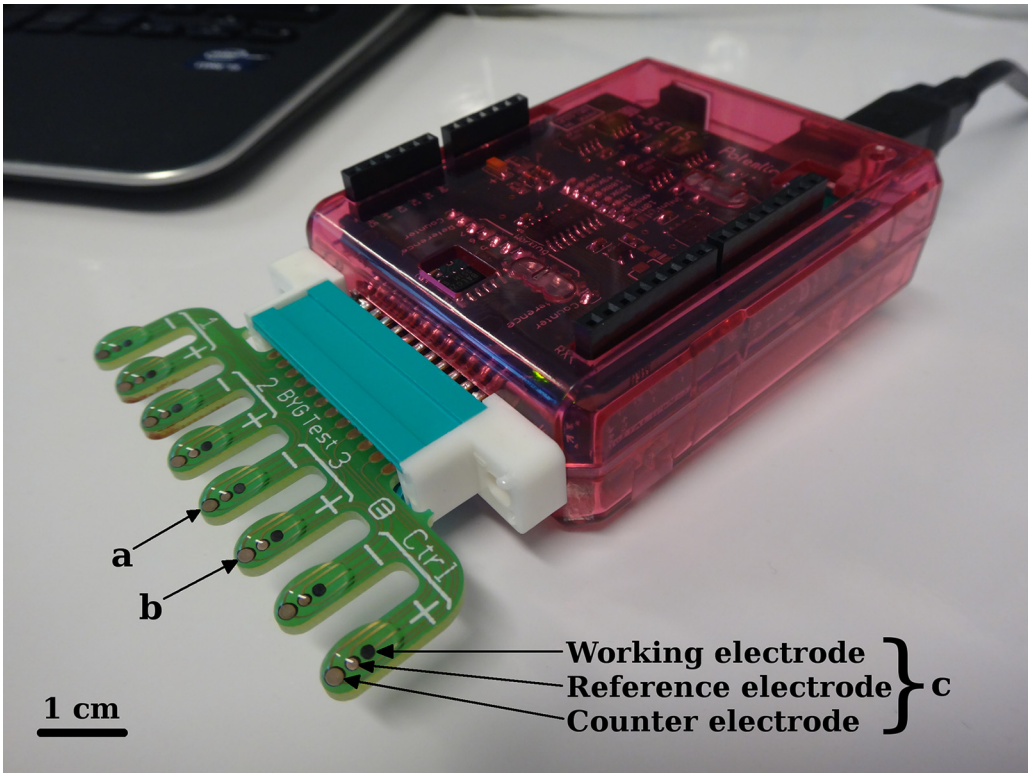

FIG 1 Homemade potentiostat and eight-probe disposable electrode. Four isolates were analyzed in parallel (numbers 1 to 3 and one control [Ctrl]). Fifty-microliter drops of bacterial suspension in lysis buffer with (+) or without (−) imipenem were loaded on fingers corresponding to the isolates to be analyzed. Probe a was loaded with a drop of strain 3 suspended in buffer without imipenem; probe b was loaded with a drop of strain 3 suspended in buffer with imipenem; probe c was comprised of one working, one reference, and one counter electrode. The software subtracts the data obtained without imipenem from the data obtained with imipenem and generates a resulting real-time curve imaging the conductance of the polyaniline.

extract of CPE (13, 14). Since then, several investigators have improved the sensitivity of the method as well as the interpretation of the results (15–19).

Recently, Nordmann and colleagues developed a colorimetric method for the specific detection of carbapenemase activity, the Carba NP test (6). In this test the pH decrease concomitant with the hydrolysis of imipenem by a carbapenemase is monitored by phenol red, which acts as an acid-base colorimetric indicator. Phenol red color changes are estimated by the naked eye of the operator but are not easily traceable in the current laboratory information management systems (LIMS). The original method allows the detection of carbapenemase-producing organisms in a maximum of 2.5 h and is now commercialized by bioMérieux (Rapidec Carba NP).

In the present paper, we propose the BYG (Bogaerts-Yunus-Glupczynski) Carba test, a new and original electrochemical method for the rapid confirmation of carbapenemase-producing bacteria. The BYG Carba test detects the variations of conductivity of a polyaniline (an electro-sensing polymer)-coated electrode which is highly sensitive to the modifications of pH and of redox activity occurring during the imipenem enzymatic hydrolysis reaction (20–22). The modifications of the conductivity are monitored, analyzed, and reported in real time by the BYG Carba test and are indicative of the presence of a carbapenemase.

MATERIALS AND METHODS

Electrochemical instrumentation. The portable potentiostat is a homemade electronic device (23), computer controlled via a USB serial port of a microcontroller board (Arduino uno interface) programmed as a control and acquisition card.

The potentiostat is multiplexed and analyzes eight different probes in parallel (Fig. 1). The instrument has a size of about 75 mm by 55 mm by 20 mm.

The electrodes. The system uses disposable electrodes of eight probes that are produced by classical printed circuit board (PCB) realization techniques. The copper circuitry is protected by a solder mask varnish.

As polyaniline cannot be electro-synthesized on copper and as copper can be easily oxidized, all the electrode areas are coated with a screen-printed carbon layer having a resistance of approximately 14 to 20 Ω/square at a 25-μm dry film thickness.

Individual probes are composed of three electrode round spots (Fig. 1). The top spot has a diameter of 1 mm and constitutes the working electrode on which polyaniline is electro-synthesized. The middle electrode is the reference electrode; it has a diameter of 1 mm and is functionalized by applying a small spot of solid Ag/AgCl amalgam (Dupont 5874 silver/silver chloride composition; 4 h of curing at 80°C) on top of the carbon layer. This solid Ag/AgCl reference electrode has been checked for its stability, repeatability, and reliability in different measurement setups from pH 2 to 12. This reference displays an electrode potential 100 mV higher (+300 mV versus a standard hydrogen electrode [SHE]) than that of a commercial Ag/AgCl reference electrode (+197 mV versus SHE). The bottom electrode has a diameter of 1.5 mm and constitutes the counter electrode. It has a bigger surface and is also covered with the Ag/AgCl

amalgam in order to prevent it from being the current limitation against the working electrode.

Each of the eight probes of the prepared electrodes is assignable by multiplexers present on the potentiostat card. The reference and counter electrodes are common between probes regarding the potentiostat electronic circuit. The electrode probes are about 4 mm.

Polyaniline electro-polymerization is performed by using the potentiostat in coulometry on the eight electrode probes placed in a row of a 96-multiwell platform. Each cell is filled with 300 μl of a 0.2 M aniline–2 M HCl aqueous solution. Electro-polymerization is performed up to 60 μC of charge on each working electrode at 890 mV against the solid Ag/AgCl reference electrode. After electro-synthesis, the electrode's probes are rinsed three times with distilled water, twice with 1 M aqueous ammonia, and finally three more times with distilled water. The electrodes are then dried using N_2 and stored in common 96-multiwell platforms before any subsequent test. Aniline was distilled under reduced pressure prior to any experimentation. All chemicals were purchased from Sigma-Aldrich (Diegem, Belgium). Electro-synthesis and measurements were all performed at room temperature (20° to 25°C).

Conductometry. The electrochemical equilibrium potential of the polyaniline electrode is first determined using the potentiostat and a method described previously (22, 23). The electrode is then brought to 10 mV above the equilibrium potential, and the current transient response is measured at a 34,487-Hz sampling rate during 11.6 ms (400 measured values). The electrode is then reset to its first equilibrium potential during 1 s. Finally, the electrode is brought to 10 mV below the equilibrium potential, and the current transient response is measured again at a 34,487-Hz sampling rate during 11.6 ms (400 measured values). Generally, the two measured current decays are mirror images, and their absolute values can be averaged. This decay is then integrated over the 400 measured values in order to obtain an arbitrary unit value that reflects the polyaniline electrochemical cell conductivity. This entire procedure is repeated over time whenever a stable equilibrium potential is attained during the hydrolysis measurement.

In a typical measurement, this type of signal, representing the conductance of the polyaniline and expressed in arbitrary units (AU), is recorded simultaneously for the four isolates that can be analyzed simultaneously on the electrode of the BYG test during 30 min. For each tested strain, two probes are used (Fig. 1, strains 1, 2, and 3 and a control strain, 4); one probe measures the signal with imipenem and the second probe measures the signal without imipenem (Fig. 1, + and −, respectively).

BYG Carba test and Carba NP test. For the BYG Carba test, a full 10-μl loop of bacteria recovered from a fresh 18- to 24-h culture on tryptic soy agar (TSA)-sheep blood agar (Becton Dickinson, Erembodegem, Belgium) is suspended in 100 μl of an in-house prepared lysis buffer. Thirty microliters of this bacterial suspension is mixed with 100 μl of a 0.1 mM $ZnSO_4$ solution with or without 3 mg/ml imipenem (6 mg/ml consisting of 3 mg/ml imipenem monohydrate plus 3 mg/ml cilastatin sodium [Tienam]; MSD France, Courbevoie, France). A 50-μl aliquot of this suspension is transferred on the probes (Fig. 1). A signal cutoff of 3.5 (arbitrary units) was chosen for the discrimination between carbapenemase and non-carbapenemase producers The results of the BYG Carba test are visualized as a curve appearing in real time. One curve is obtained for the signal (conductance) detected with imipenem, and another curve is obtained for the signal without imipenem (background curve). The software then subtracts the background from the signal obtained with imipenem (data not shown). Once the resulting curve crosses the cutoff, the corresponding isolate is reported as positive. At the end of the run, the software generates a report (Fig. 2). A preliminary control without bacterial extract was performed to confirm the stability of the imipenem in the solution during the experiment (data not shown).

For comparison, the Carba NP test was performed in parallel to the BYG Carba test on the same bacterial culture isolates according to the procedure initially published by Nordmann et al. (6).

Bacterial isolates. The BYG Carba test was initially validated against a collection of 57 *Enterobacteriaceae* isolates previously characterized for their resistance mechanisms to β-lactam agents (24–26). These isolates comprised 41 carbapenemase producers and 16 noncarbapenemase producers (Table 1). The BYG Carba test was subsequently challenged against an external quality control (EQA) proficiency testing panel consisting of 10 putative CPE isolates (EQA exercise 2013, European survey on CPE [EuSCAPE] project; University Medical Center Groningen [UMCG] in collaboration with the United Kingdom National External Quality Assessment Service [NEQAS], September 2013) (Table 2). Imipenem, meropenem, and ertapenem MICs were determined by Etest (bioMérieux, Marcy l'Etoile, France) according to Clinical and Laboratory Standards Institute (CLSI) guidelines (27).

The performance of the test was also assessed prospectively on 324 consecutive *Enterobacteriaceae* isolates referred to the National Reference Laboratory (NRL) by Belgian laboratories in 2014 for nonsusceptibility to at least one carbapenem (ertapenem, imipenem, or meropenem). Nonsusceptibility to carbapenems was assessed by the referring laboratories according to CLSI (27) or to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org), which are the two sets of guidelines used by most microbiologists in Belgium. Bacterial confirmation of identification to the species level was carried out centrally at the NRL using matrix-assisted laser desorption–ionization time of flight (MALDI-TOF) mass spectrometry on a Microflex LT (Bruker Daltonics GmbH, Bremen, Germany), and antimicrobial resistance profiles were analyzed phenotypically by a disk diffusion method according to CLSI guidelines (27). Meropenem and ertapenem MICs were determined using broth microdilution panels (Sensititre, Thermo Fisher Scientific, Cleveland, IL, USA) in the prospective study.

All isolates were verified for the presence of carbapenemase by in-house multiplex PCR targeting bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{KPC}, and bla_{OXA-48} (26) and other β-lactamase genes (24, 25). Results of the molecular tests were considered the gold standard for the presence of a carbapenemase. In the case of discrepancies observed between hydrolysis tests and PCR results, the resistance genes detected by PCR were sequenced using an external service company (Macrogen, Inc., Seoul, South Korea). The sequence obtained was compared with the genes present in GenBank and aligned with the reference gene referred at the Lahey clinic (http://www.lahey.org/Studies/).

RESULTS

Validation of collection isolates and external quality assessment. Of the 57 collection isolates tested in triplicate (Table 1), the BYG Carba test correctly and repeatedly detected 40 of the carbapenemase producers but missed one single GES-6-producing isolate, a very weak and rarely reported class A serine carbapenemase (28). On the other hand, all noncarbapenemase producers yielded a negative BYG Carba test result. Based on these results, the BYG Carba test was found to display a sensitivity of 97.6% and a specificity of 100%. All 40 CPE isolates were detected as positive by the BYG Carba test within 15 min, with 34 CPE isolates being detected in less than 5 min (including 9 out of 12 OXA-48-like producers). No correlation between the intensity of the signal and MICs could be observed (Table 1). The maximum observed signal value after 30 min of reaction was 70.2 (arbitrary units [AU]) for an NDM-1-producing *K. pneumoniae* isolate, and the minimum was 9.9 AU for a VIM-1-producing *Citrobacter braakii* (data not shown).

The BYG Carba test was further validated with an external quality control provided by the NEQAS which distributed a panel of 10 suspected CPE isolates during September 2013 (Table 2). The agreement with expected results was 100%. The BYG Carba test correctly identified the 9 CPE isolates and the single non-carbapenemase-producing *Enterobacter aerogenes*. Eight out of

BYG Test Report

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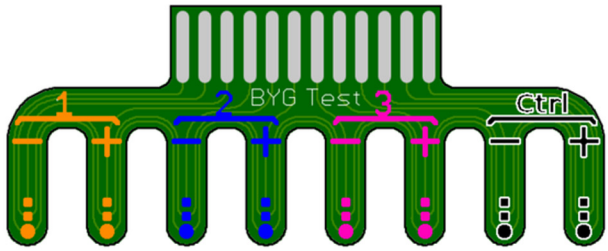

Strain 1 : NEQAS 1943 (POSITIVE)
Strain 2 : NEQAS 1945 (POSITIVE)
Strain 3 : CNR20150311 (NEGATIVE)
Strain 4 (Ctrl) : CNR20150325 (POSITIVE)

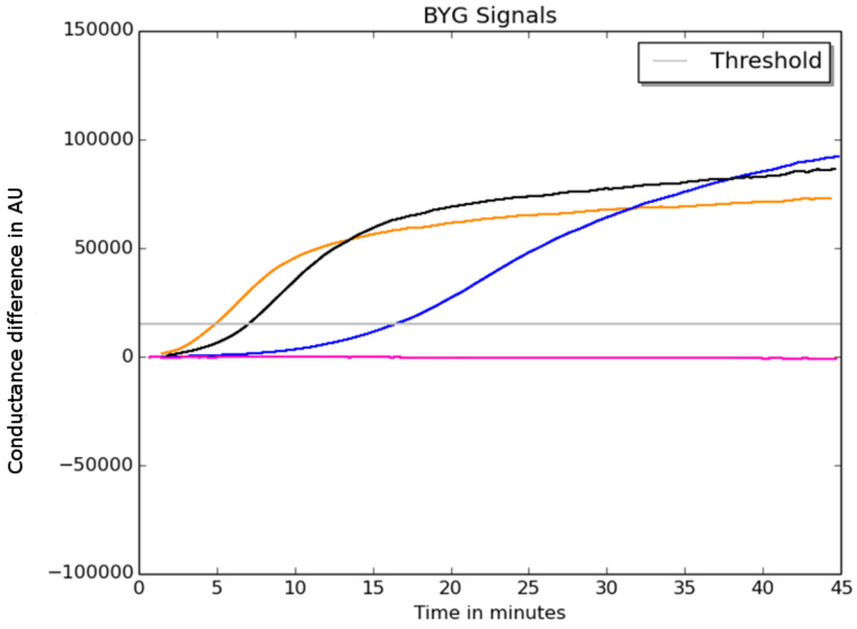

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FIG 2 Real-time curve obtained with the BYG Carba test. A report of the results automatically generated by the software is shown. The gray horizontal line represents the cutoff line. The colored lines represent the real-time curve generated during the analysis. The orange (*K. pneumoniae* OXA-48 NEQAS 1943), blue (*K. pneumoniae* VIM-1 NEQAS 1945), and black (*K. pneumoniae* OXA-48 control strain CNR20150325) curves correspond to positive strains, and the pink flat curve corresponds to a negative *Enterobacter aerogenes* strain (CNR 20150311). The *y* axis is linked to the conductance of polyaniline, and values are expressed in arbitrary units.

TABLE 1 Validation of the BYG Carba test on 57 collection isolates

Carbapenemase and isolate (*n*)[a]	MIC (μg/ml) of:			BYG Carba result[b]	BYG signal (AU)[c]	SD	Time to result (min)
	Imipenem	Meropenem	Ertapenem				
OXA-48-like (12)							
OXA-48 (11)							
Klebsiella pneumoniae (5)	0.19 to 1	0.38 to 3	0.75 to 2	POS	22.2 to 30.8	1.5 to 5.5	<5 to 15
Escherichia coli (2)	0.75, 4	1.5, 8	1.5, 8	POS	33.2 to 35.5	4.8, 4.9	<5
Citrobacter freundii (1)	1.5	0.75	3	POS	35.8	4.6	<5
Enterobacter cloacae (2)	0.75, 1.5	0.38, 0.75	4, 12	POS	27.7, 31.2	1.2, 3.3	<5
Klebsiella oxytoca (1)	0.5	3	1.5	POS	31.2	0.9	>5 to 10
OXA-162 (1)							
Klebsiella pneumoniae (1)	0.75	0.38	24	POS	23.5	4.5	>5 to 10
KPC (8)							
KPC-2 (8)							
Klebsiella pneumoniae (8)	8 to >32	6 to >32	8 to >32	POS	34.5 to 53.9	0.9 to 9.9	>5 to 10
NDM (8)							
NDM-1 (7)							
Enterobacter cloacae (1)	3	4	4	POS	35.6	2.8	<5
Escherichia coli (2)	4	0.75 to >32	3 to >32	POS	32.2 and 51.4	2.7 and 6.3	<5
Klebsiella pneumoniae (3)	2 to >32	4 to >32	12 to >32	POS	50.6 to 65.6	2.1 to 6.0	<5
Morganella morganii (1)	24	6	1	POS	35.6	2.8	<5
NDM-5 (1)							
Escherichia coli (1)	16	>32	>32	POS	32.2	2	<5
VIM (8)							
VIM-1 (4)							
Citrobacter braakii (1)	3	1.5	2	POS	14.2	5.1	<5
Klebsiella pneumoniae (1)	32	24	16	POS	29.2	0.7	>5 to 10
Klebsiella oxytoca (1)	24	2	8	POS	35	3.4	<5
Providencia vermicola (1)	>32	>32	2	POS	30.8	8.4	>5 to 10
VIM-4 (2)							
Serratia marcescens (1)	32	>32	32	POS	30.4	1.7	>5 to 10
Aeromonas caviae (1)	0.75	0.064	0.34	POS	50.6	0.8	<5
VIM-27 (1)							
Klebsiella pneumoniae (1)	>32	>32	>32	POS	35.3	5.9	<5
VIM-31 (1)							
Enterobacter cloacae (1)	1.5	1.5	0.5	POS	41.7	9.9	<5
IMP (3)							
IMP-4 (1)							
Klebsiella pneumoniae (1)	1	1.5	1.5	POS	42.7	3.3	<5
IMP-8 (1)							
Enterobacter cloacae (1)	12	3	3	POS	39.3	3.8	<5
IMP-11 (1)							
Serratia marcescens (1)	3	2	3	POS	44.3	6.2	<5
GIM (1)							
GIM-1 (1)							
Enterobacter cloacae (1)	0.38	1.5	3	POS	61.1	4.5	<5
GES (1)							
GES-6 (1)							
Citrobacter braakii (1)	0.5	0.094	0.064	NEG	−1.60	0.62	
No carbapenemase (16)[d]							
Citrobacter amalonaticus (1)	0.19	0.047	0.016	NEG	−3.3	1.8	30
Citrobacter freundii (1)	0.25	0.19	0.38	NEG	−0.1	0.5	30
Enterobacter aerogenes (1)	0.25	0.064	0.25	NEG	−0.8	0.6	30
Enterobacter asburiae (1)	0.19	0.125	0.19	NEG	0.2	0.7	30
Escherichia coli (4)	0.125 to 0.19	0.032 to 0.23	0.012 to 0.032	NEG	−15.3 to 0.2	0.4 to 10.8	30
Klebsiella pneumoniae (4)	0.125 to 0.25	0.047 to 2	0.016 to 0.75	NEG	−1.6 to 0.1	0.2 to 1.9	30
Proteus mirabilis (1)	1.5	0.19	0.125	NEG	−0.1	0.9	30
Providencia stuartii (1)	0.5	0.125	0.023	NEG	−4.6	1.9	30
Salmonella sp. (1)	0.25	0.094	0.047	NEG	−1,5	1.5	30
Serratia marcescens (1)	0.25	0.094	0.125	NEG	−0.7	0.4	30

[a] *n*, number of isolates.
[b] POS, positive; NEG, negative.
[c] AU, arbitrary units.
[d] Including the following: TEM-1 (*n* = 9), TEM-24 (*n* = 1), TEM-30 (*n* = 1), SHV-1 (*n* = 1), SHV-2a (*n* = 2), SHV-11 (*n* = 1), SHV-12 (*n* = 2), SHV-28 (*n* = 1), SHV-76 (*n* = 1), CTX-M-2 (*n* = 1), CTX-M-3 (*n* = 1), CTX-M-14 (*n* = 1), CTX-M-15 (*n* = 3), GES-7 (*n* = 1), OXA-1 (*n* = 1), OXA-10 (*n* = 1), OXA-163 (1), ACC-1 (*n* = 1), DHA-1 (*n* = 1), DHA-7 (*n* = 1), and extended-spectrum AmpC (*n* = 1).

TABLE 2 Results of the BYG Carba Test on the carbapenemase-producing *Enterobacteriaceae* panel NEQAS 2013

Specimen no.	Species	Carbapenemase	MIC (μg/ml) of:			BYG Carba result[a]	Time to result (min)	BYG signal (AU)[b]
			Imipenem	Meropenem	Ertapenem			
NEQAS 1940	*K. pneumoniae*	KPC	>32	>32	>32	POS	<5	64.3
NEQAS 1941	*E. cloacae*	NDM-1	>32	>32	>32	POS	<5	50.9
NEQAS 1942	*K. pneumoniae*	KPC-3	>32	>32	>32	POS	<5	62.8
NEQAS 1943	*K. pneumoniae*	OXA-48	1	1.5	4	POS	<5	38.2
NEQAS 1944	*K. pneumoniae*	KPC-2	>32	>32	24	POS	<5	54.9
NEQAS 1945	*K. pneumoniae*	VIM-1	>32	>32	>32	POS	>5–10	32.7
NEQAS 1946	*K. pneumoniae*	NDM-1	>32	>32	>32	POS	<5	50.3
NEQAS 1947	*K. pneumoniae*	IMP-1	12	3	8	POS	<5	61.3
NEQAS 1948	*K. pneumoniae*	NDM-1	>32	>32	>32	POS	<5	58.3
NEQAS 1949	*E. aerogenes*	None	6	3	32	NEG	30	1.0

[a] S, positive; NEG, negative.
[b] Arbitrary unit.

the nine CPE isolates were detected in less than 5 min, including one OXA-48-producing *K. pneumoniae* isolate (NEQAS 1943), while the VIM-1-producing *K. pneumoniae* isolate (NEQAS 1945) was detected between 5 and 10 min after the start of the test. The maximum value (64.3) was observed for a KPC producer (NEQAS 1940), and the lowest (32.7) was obtained for the VIM-1 producer (NEQAS 1945). The value at 30 min for the non-carbapenemase-producing *E. aerogenes* isolate (NEQAS 1949) was 1.0.

Prospective evaluation. The BYG Carba test was then evaluated prospectively on consecutive strains referred to the NRL for the detection of CPE (Table 3).

Over a 4-month period during the first half of 2014, 324 suspected CPE isolates collected in 66 Belgian laboratories were received by the NRL. All isolates were analyzed for carbapenem susceptibility testing by the Carba NP test, by the BYG Carba test, and by multiplex PCR for the identification of carbapenemase genes according to the procedures described in Materials and Methods. All 324 *Enterobacteriaceae* isolates could be analyzed and yielded an interpretable result with the BYG Carba test while the Carba NP test gave definite results for 313 isolates (96%) and no result for 11 (3.4%) strains (7 mucoid isolates could not be sampled by pipetting because of hyperviscosity after the lysis step of the Carba NP test and 4 isolates had noninterpretable results, with the control without imipenem yielding an orange color not distinguishable from that of the strain with imipenem). Among the 11 isolates with an indeterminate Carba NP test result (*K. pneumoniae*, $n = 5$; *Enterobacter cloacae*, $n = 2$; *Citrobacter freundii*, $n = 2$; *Escherichia coli* and *Klebsiella oxytoca*, $n = 1$ each), two isolates produced an OXA-48 and were positive with the BYG test, and nine were non-carbapenemase producers with a negative BYG test result.

In comparison with results from PCR, considered the reference gold standard, the BYG Carba test gave 315/324 (97.2%) interpretable and correct results after 30 min, and the Carba NP test yielded 294/324 (90.7%) interpretable and correct results after 2.5 h (including lysis). Neither the BYG Carba nor Carba NP test presented false-positive results (100% specificity for each of the tests).

Nine strains out of 178 carbapenemase producers (5.1%) yielded a false-negative result with the BYG Carba test (OXA-48, $n = 7$; OXA-232, $n = 1$; and VIM-2, $n = 1$), while 19 false-negative results (10.7%) occurred with the Carba NP test (OXA-48, $n = 16$, and OXA-232, $n = 3$). Four OXA-48-like producing *K. pneumoniae* isolates (OXA-48, $n = 3$, and OXA-232, $n = 1$) were not detected by any of the tests (Table 4).

Overall, the BYG Carba test yielded sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPP) of 95, 100, 100, and 94%, respectively, and the values obtained with the Carba NP test were 89, 100, 100, and 88%, respectively, not taking into account the 11 noninterpretable results.

One definite advantage of the BYG Carba test is related to the fact that once a cutoff limit is set, the interpretation becomes objective by reporting of a number. With the current method of analysis, for the 169 isolates (out of 178 CPE isolates) with a positive BYG Carba test result, the values obtained ranged between 3.63 and 76.83 AU (mean, 32.08 AU; median, 31.34 AU; standard deviation [SD], 16.50 AU). For the 155 BYG-negative strains including 146 non-CPE isolates, the values of the BYG signal ranged between −29.91 and 3.17 AU (mean, −1.19 AU; median, −0.20 AU; SD, 4.1 AU), with a negative value indicating that, after 30 min, the sample without imipenem presented a higher value than the sample with imipenem. The highest values were observed for KPC producers (mean, 52.25 AU; SD, 12.07 AU) followed by NDM producers (mean, 50.25 AU; SD, 7.45 AU), VIM producers (mean, 30.78 AU; SD, 10.35 AU), and OXA-48 producers (mean, 22.16 AU; SD, 12.08 AU) (Table 3).

Regarding the time to result, 90 out of 178 (50.6%) of the carbapenemase producers were detected in less than 5 min (including 40 OXA-48 producers), and more than 80% of the CPE isolates (144/178) were detected in less than 15 min (data not shown). No correlation could be observed between meropenem or ertapenem diameter or MIC and the intensity of the signal.

DISCUSSION

The detection of CPE is challenging for diagnostic microbiological laboratories (10) but is of utmost importance for prevention and outbreak control in clinical settings (29). Rapid, sensitive, and specific laboratory detection techniques based on the detection of carbapenem hydrolysis have been recently proposed in the literature (10). The most widespread colorimetric test, the Carba NP test, was reported by several laboratories as lacking some degree of sensitivity, especially for the detection of OXA-48 and for some metallo-β-lactamase producers (30–34). Several groups or companies subsequently developed slightly modified tests based on the principle of the Carba NP test. For

TABLE 3 Prospective evaluation of the BYG Carba test on 324 isolates

Carbapenemase, isolate, and prospective result (*n*)[a]	Meropenem MIC (μg/ml)	Ertapenem MIC (μg/ml)	BYG Carba result[b]	BYG signal (AU)[c]	Time to result (min)	Carba NP result (no. of isolates and appearance)
OXA-48 (117)						
BYG positive (109)						
Klebsiella pneumoniae (65)	<0.25 to >32	<0.25 to >32	POS	3.6 to 42	<5 to 30	52 POS (50 orange, 2 yellow), 12 NEG, 1 noninterpretable
Escherichia coli (15)	<0.25 to 32	<0.25 to >32	POS	13.4 to 43.8	<5 to 20	12 POS (orange), 3 NEG
Citrobacter freundii (14)	0.5 to 8	0.5 to 2	POS	27 to 46	<5	13 POS (12 orange, 1 yellow), 1 mucoid
Enterobacter cloacae (9)	<0.25 to 4	2 to 16	POS	25.7 to 36.9	<5 to 10	9 POS (7 orange, 2 yellow)
Klebsiella oxytoca (5)	<0.25 to 2	0.5 to 4	POS	19.3 to 31.3	<5 to 15	5 POS (3 orange, 2 yellow)
Serratia marcescens (1)	>32	>32	POS	26.3	<10	1 POS (orange)
BYG false negative (8)						
Klebsiella pneumoniae (7)	<0.25 to >32	2 to >32	NEG	−1 to 2.2	30	3 POS (orange), 4 NEG
Escherichia coli (1)	<0.25	<0.25	NEG	0.4	30	1 NEG
KPC (25)						
BYG positive (25)						
Klebsiella pneumoniae (25)	2 to >32	8 to >32	POS	40.2 to 76.8	<5	25 POS (23 yellow, 2 orange)
NDM (23)						
BYG positive (23)						
Enterobacter cloacae (13)	4 to >32	8 to >32	POS	35.9 to 59.0	<5	13 POS (11 yellow, 2 orange)
Escherichia coli (4)	2 to 16	4 to 16	POS	45.9 to 56.6	<5 to 10	4 POS (3 yellow, 1 orange)
Klebsiella pneumoniae (3)	8 to >32	16 to >32	POS	43.5 to 63.7	<5	3 POS (1 yellow, 2 orange)
Aeromonas caviae (1)	2		POS	49.7	<10	1 POS (orange)
Citrobacter freundii (1)	4	8	POS	56.2	<5	1 POS (yellow)
Klebsiella oxytoca (1)	32	32	POS	53.8	<5	1 POS (yellow)
VIM (13)						
BYG positive (12)						
Enterobacter cloacae (4)	4 to 32	8 to 32	POS	38.4 to 58.3	<5 to 10	4 POS (3 yellow, 1 orange)
Klebsiella pneumoniae (3)	<0.25 to 1	<0.25 to 2	POS	10.3 to 32.6	>5 to 15	3 POS (3 yellow)
Escherichia coli (2)	<0.25 and 1	<0.25 and 1	POS	10.6 and 19.1	20 and 25	2 POS (2 yellow)
Citrobacter amalonaticus (1)	16	8	POS	38.8	<5	1 POS (yellow)
Citrobacter freundii (1)	8	8	POS	32.9	<5	1 POS (orange)
Klebsiella oxytoca (1)	>32	32	POS	22.1	<5	1 POS (yellow)
BYG false negative (1)						
Klebsiella oxytoca (1)	32	>32	NEG	1.6	30	1 POS (yellow)
No carbapenemase (146)[d]						
BYG negative (146)						
Klebsiella pneumoniae (51)	<0.25 to 32	<0.25 to >32	NEG	−19.4 to 3.2	30	47 NEG, 3 noninterpretable, 1 mucoid
Enterobacter cloacae (34)	<0.25 to 2	<0.25 to 16	NEG	−20.2 to 1.3	30	32 NEG, 2 mucoid
Citrobacter freundii (3)	<0.25 to 4	1 to 16	NEG	−29.9 to −0.2	30	2 NEG, 1 mucoid
Enterobacter aerogenes (21)	<0.25 to 16	<0.25 to >32	NEG	−1.07 to 1.8	30	3 NEG
Escherichia coli (24)	<0.25 to 4	<0.25 to 32	NEG	−17.2 to 0.9	30	23 NEG, 1 mucoid
Klebsiella oxytoca (6)	<0.25 to 8	1 to 16	NEG	−1.4 to 2.8	30	5 NEG, 1 mucoid
Morganella morganii (2)	<0.25	<0.25	NEG	−12.25 and 1.8	30	2 NEG
Proteus mirabilis (2)	<0.25 and 1	<0.25	NEG	−2.9 and −0.5	30	2 NEG
Serratia marcescens (2)	<0.25 and 2	1 and 16	NEG	0.2	30	2 NEG
Citrobacter braakii (1)	<0.25	1	NEG	−0.2	30	1 NEG

[a] *n*, number of isolates.
[b] POS, positive; NEG, negative.
[c] AU, arbitrary units.
[d] Including the following: TEM (*n* = 28), SHV (*n* = 30), OXA-1 group (*n* = 29), CTX-M of group 1 (*n* = 22), CTX-M of group 2 (*n* = 1), CTX-M of group 9 (*n* = 1), and DHA (*n* = 9).

example, Pires et al. proposed and evaluated a Carba NP-like test using bromothymol blue instead of the phenol red as a pH indicator with 93.3 to 100% sensitivity and 100% specificity (7, 35). The Rosco Diagnostica company (Taastrup, Denmark) developed two distinct commercial tests (Rosco Rapid Carb screen kits), also based on the hydrolysis of imipenem using either phenol red or bromothymol blue as the indicator. The published evaluation of this test revealed some difficulty in obtaining readings, resulting in up to 12% of noninterpretable results and poor specificity (83%) (8, 36). A recent evaluation

TABLE 4 False-negative and discrepant results between the BYG Carba and the Carba NP tests

OXA-48-like discrepant result and isolate (*n*)[a]	Meropenem MIC (μg/ml)	Ertapenem MIC (μg/ml)	BYG Carba result[b]	BYG signal (AU)[c]	Time to result (min)	Carba NP result (color)
False-negative Carba NP only (15)						
K. pneumoniae OXA-48 (10)	<0.25 to 1	1 to 8	POS	5.4 to 34.4	30	NEG
K. pneumoniae OXA-232 (2)	16 and >32	>32	POS	4.1 and 4.4	30	NEG
E. coli OXA-48 (3)	<0.25 to 32	<0.25 to >32	POS	22.8 to 27.5	<5 to 15	NEG
False-negative BYG Carba only (5)						
K. pneumoniae OXA-48 (3)	1 to >32	4 to >32	NEG	1.5 to 2.1	30	POS (orange)
E. coli (1)	<0.25	<0.25	NEG	0.4	30	POS (orange)
K. oxytoca VIM-2 (1)	32	>32	NEG	1.6	30	POS (yellow)
False-negative BYG Carba and Carba NP (4)						
K. pneumoniae OXA-48 (3)	<0.25 to 4	2 to 32	NEG	−1.0 to 0.5	30	NEG
K. pneumoniae OXA-232 (1)	>32	>32	NEG	2.2	30	NEG

[a] *n*, number of isolates. Total number of isolates, 23.
[b] POS, positive; NEG, negative.
[c] AU, arbitrary units.

of Dortet and colleagues further confirmed the poor specificity of this test (71%) (37).

Very recently, bioMérieux proposed a commercial ready-to-use version of the Carba NP test, the Rapidec Carba NP. The test still requires an incubation time of 30 min before incubation of the plastic tray at 37°C and interpretation of the results after 30, 60, and 120 min. Two evaluations of the test by the inventors revealed sensitivity and specificity varying between 96 and 99% and between 96 and 100%, respectively (37, 38). All of these tests have in common the fact that they are interpreted by naked eye (color modification), and hence readings may be subjective and variable between observers, especially when color changes are very faint.

In this article, we propose the BYG Carba test, a novel original electrochemical diagnostic tool for the rapid detection of CPE from bacterial suspensions.

The BYG Carba test indeed offers several advantages in comparison to colorimetrically based methods. First, the BYG technology reduces the time to results to about 30 min, and the test can be performed at room temperature without incubation at 37°C. Second, the BYG method takes its advantages from the fact that, in addition to the acidification of the medium, the oxydo-reduction process also participates in the signal detected by the system (20, 21), suggesting that the BYG test could be a better sensor for the hydrolysis of carbapenemases than only a colorimetric pH indicator or iodine indicators (39). In our hands, the test leads to a significant improvement for the detection OXA-48 producers compared to results with the Carba NP test (8 false-negative results for the BYG Carba test versus 19 with the Carba NP test; $P < 0.02$). However, this could be, at least partially, the consequence of the subjective analysis of the Carba NP test as Papagiannitis and colleagues recently showed that interpretation of the Carba NP test by an enzyme-linked immunosorbent assay (ELISA) reader improves the sensitivity of the test from 76 to 81% (18). In our hands the sensitivity for OXA-48-like detection by the Carba NP test was 83.8%, and with the BYG Carba test it was 93.2%.

Third, an electrochemical test permits the real-time objective measurement and the traceability of the signal, with the results being visualized as a real-time curve (Fig. 2). In the scope of the accreditation process for the clinical laboratory, this element may represent a substantial advantage. During the prospective evaluation, definitive positive results (i.e., production of carbapenemase) could be confirmed in less than 5 min in more than 50% of the cases. Moreover, in the current format, the BYG Carba test can analyze up to four strains in parallel, but the technology also allows parallel arrangement of the electrodes, allowing testing of up to 192 strains (48 electrodes). The electrodes are currently produced in the laboratory at about $2/electrode and $0.50/strain. The reader is also produced in-house (about $100), and a software program automatically interprets the data and generates a report (pdf format). In conclusion, the BYG Carba test is a novel electrochemical assay that was developed for the detection of CPE. This method is rapid (detection within 30 min), traceable, objective, sensitive, and specific, with slightly improved performance compared to that of the Carba NP test. Further evaluation of this test by independent investigator groups is currently ongoing.

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