Product Description

An innovative in vitro diagnostic for the rapid quantitative determination of β-lactamase activity.
VALUE PROPOSITION OF BIOLACTAM®

1. SIMPLE PROCEDURE

NO NEED FOR BACTERIAL CULTURES

NO MICROBIOLOGICAL LABORATORY NEEDED

2. BETTER DIAGNOSTICS

Determination of combined beta-lactamase activity

Quick results: 1.5 to 3 hours

3. IMPROVED TREATMENT PLAN

BIOLACTAM® IS A NEW TEST SYSTEM FOR THE DETECTION OF BETA-LACTAMASE ACTIVITY WITH OUTSTANDING BENEFITS AGAINST STANDARD METHODS

FEATURES OF BIOLACTAM

BIOLACTAM®

In-vitro diagnostic for the detection and quantification of beta-lactamase activity in biological fluids (serum, oral fluid, cerebrospinal fluid, urine) and bacterial suspensions

- Detects microbiological resistance to beta-lactam antibiotics
- Alternative to the beta-lactam detection methods in clinical practice

ADVANTAGES

- Does not demand a culture of bacteria
- No bacteriological laboratory needed
- Quick results (1.5 to 3 hours)
- Determination of combined beta-lactamase activity

COMPONENTS

- Lyophylized nitrocefin (chromophore)
- Penicillinase (enzyme)
- Phosphate buffer (isotonic solution)
Biolactam® offers unique advantages over the identified substitution tests performed in clinical laboratories

- Laboratory equipment and staff needed to perform the test
- Expertise in handling tests and interpretation of results
- Costs of performing the test
- Breeding of bacterial cultures

Influencing factors

Biolactam’s added value lies in the short duration as well as the low effort level to perform antibiotic resistance tests

Clinical significance of beta-lactam antibiotics and resistance situation

Volume of prescription

- Beta-lactam based antibiotics account for approx. 50% of antibiotic prescriptions in Germany
- Penicillins followed by cephalosporins are the most widely prescribed antibiotics
- Hospitals account for almost 14% of overall antibiotic prescriptions
- Antimicrobial resistance is one of our most serious health threats
- 26,000 drug-resistant infections due to extended spectrum beta-lactamase (ESBL) producing enterobacteriaceae in the U.S.

Target-directed therapy in knowledge of the status of resistance in infected patients is required

Sources:
3. CDC (2013) ANTIBIOTIC RESISTANCE THREATS in the United States, pp 1-114

Own illustration; Prof. Dr. Rodloff (2013) | *Agent is unknown before testing
ASSAY PRINCIPLE AND FIELD OF USE

ASSAY PRINCIPLE STEPS

1. Transfer biological sample, e.g. blood serum, sputum, cerebrospinal fluid, and dissolved nitrocefin to multiwell plate
2. Mix the compound
3. Incubate at 37 degrees for 30 to 120min
4. Measure optical density on microplate reader at 492nm (bathochromic shift of hydrolyzed nitrocefin)

FIELD OF USE

- Respiratory tract infections
- CNS infections
- Surgical infections and pre-operative treatment (surgical departments)
- Severe bacterial infections with systemic lesions (intensive care units)
- ENT infections
- Dental infections
- Urinary infections
- Gynecological infections

BIOLACTAM® IS A MECHANISM-SPECIFIC TEST HAVING ADVANTAGES OVER ALTERNATIVE METHODS, PROVIDING QUANTITATIVE THRESHOLD VALUES IN A SHORT TIME

COMPARISON OF TEST METHODS FOR THE DETECTION OF β-LACTAMASE ACTIVITY

<table>
<thead>
<tr>
<th>Sensitivity Testing Method</th>
<th>Method</th>
<th>Type</th>
<th>Duration</th>
<th>Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth dilution</td>
<td>MIC</td>
<td>Quantitative</td>
<td>3 days</td>
<td>Low</td>
</tr>
<tr>
<td>Agar dilution</td>
<td>Lowest concentration at which bacteria is still inhibited</td>
<td>Quantitative</td>
<td>3 days</td>
<td>Low</td>
</tr>
<tr>
<td>Disk diffusion</td>
<td>Zone of inhibition</td>
<td>Qualitative (susceptible, intermediate, resistant)</td>
<td>1-2 days</td>
<td>Low</td>
</tr>
<tr>
<td>E-Test</td>
<td>Plastic strip/MIC</td>
<td>Quantitative/numerical scale</td>
<td>24h</td>
<td>Potentially high (separate strip for each antibiotic)</td>
</tr>
<tr>
<td>Automated antimicrobial susceptibility testing systems</td>
<td>Computer-assisted</td>
<td>Quantitative</td>
<td>4-24h (depending on system)</td>
<td>High initial costs &amp; maintenance</td>
</tr>
<tr>
<td>Mechanism-specific test BIOLACTAM®</td>
<td>Colorimetric essay detects β-lactamase activity</td>
<td>Quantitative</td>
<td>1.5 – 3h</td>
<td>Relatively low</td>
</tr>
<tr>
<td>Genotypic methods</td>
<td>Molecular technique</td>
<td>Genotypic</td>
<td>1-2 weeks</td>
<td>High testing costs</td>
</tr>
</tbody>
</table>
BIOLACTAM enables the in vitro determination of β-lactamase activity in a variety of biological samples such as blood serum, sputum, cerebrospinal fluid and bacterial suspension.

### Table 1 Reagents delivered

<table>
<thead>
<tr>
<th>Vial</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125 µg Nitrocefin (chromogenic substrate)</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.1 M Phosphate buffer (PB)</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Penicillinase 10,000 IU</td>
<td>1</td>
</tr>
</tbody>
</table>

Store all reagents at +2-8 °C; shelf life: see packaging

Additional materials required: microplate reader, clear flat-bottom 96-well plate, pipettes, thermostat/incubator

#### Preparation of solutions

- Phosphate buffer solution (PB): add 4.6 mL of distilled water (H₂O) to vial 2, dissolve carefully
- Nitrocefin stock solution (0.25 mg/mL): add 500 µL PB to vial 1, dissolve carefully (storable for 1 month at -18°C)
- Solution of penicillinase (“Penicillinase”; 100,000 U/mL): add 100 µL H₂O to vial 3, dissolve carefully

#### Determination of β-lactamase activity

(A) in blood serum:

- Add 4.0 mL PB to the nitrocefin stock solution and use within one day (“Chromogen”).
- Add the respective volumes indicated in Table 2 in order from left to right into a given well; incubate at 37°C for 30 min.

<table>
<thead>
<tr>
<th>Description</th>
<th>Blood serum (µL)</th>
<th>Distilled water (µL)</th>
<th>Chromogen (µL)</th>
<th>Penicillinase (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Sample (T)</td>
<td>20</td>
<td>-</td>
<td>180</td>
<td>-</td>
</tr>
<tr>
<td>Control (C)</td>
<td>20</td>
<td>180</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blank (B)</td>
<td>-</td>
<td>20</td>
<td>180</td>
<td>-</td>
</tr>
<tr>
<td>Positive Control (P)</td>
<td>-</td>
<td>-</td>
<td>180</td>
<td>20</td>
</tr>
</tbody>
</table>

(B) in other biological fluids or bacterial suspensions:

- Add 2.0 mL PB to the nitrocefin stock solution and use within one day (“Chromogen”).
- Add the respective volumes indicated in Table 2 in order from left to right into a given well; incubate at 37°C for 120 min.

<table>
<thead>
<tr>
<th>Description</th>
<th>Biological fluid (µL)</th>
<th>Distilled water (µL)</th>
<th>Chromogen (µL)</th>
<th>Penicillinase (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Sample (T)</td>
<td>100</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Control (C)</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blank (B)</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Positive Control (P)</td>
<td>-</td>
<td>80</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>
Measurement of optical densities (OD) and calculation of the results

Measure OD on a plate reader at 492 (or 505) nm against air. Calculate the β-lactamase activity level of the sample according to the following formula.

\[ A_{bl}[^\%] = \frac{(OD_T - OD_C) - (OD_B - OD_P)}{OD_T - OD_C} \times 100 \]

\( A_{bl} \): β-lactamase activity level in Test Samples [%]; \( OD_T \): average of optical density of Test Samples; \( OD_C \): average of optical density of Control Samples; \( OD_B \): average of optical density of Blank; \( OD_P \): average of optical densities of Positive Control; \( OD_E \): average of optical densities of Empty Wells.

Clinical assessment and therapeutic advice

- **Blood serum:** if \( A_{bl} \geq 70\% \), prescription of inhibitor-protected β-lactams or antibiotics of other pharmacological groups with similar activity spectrum is recommended.

- **Sputum:** if \( A_{bl} \geq 20.0\% \), prescription of inhibitor-protected β-lactams, cefepime, carbapenems or monobactams respectively antibiotics of other pharmacological groups with similar activity spectrum is recommended.

- **Cerebrospinal fluid:** if \( A_{bl} \geq 20.0\% \), prescription of carbapenems or cefepime respectively antibiotics of other pharmacological groups with similar activity spectrum is recommended (provided the patient does not have a subarachnoidal hemorrhage).

- **Bacterial suspension:** \( A_{bl} \geq 14.2\% \) indicates clinically significant quantity of β-lactamases associated with a significant decrease of effectiveness of first-line β-lactam antibiotics (penicillins, cephalosporins of the 1st and 2nd generation). \( A_{bl} \geq 26.5\% \) indicates resistance also to inhibitor-protected β-lactams whereas \( A_{bl} \geq 81.2\% \) indicates resistance even to cephalosporins of the 3rd generation.

Benefits of BIOLACTAM

The rapid and quantitative determination of combined β-lactamase activity (microbiological and endogenous) enables a target-directed antibiotic therapy.

Limits of the method

The test-system has some limitations in the assessment of resistance of gram-positive cocci (i.e. different strains of streptococci and staphylococci) towards β-lactam antibiotics. Although the resistance of a number of staphylococci strains to antibiotics of the β-lactam group is caused by the production of β-lactamases, all streptococci and some staphylococci strains also modify penicillin-binding proteins. Therefore, a negative test result for the bacterial suspension does not preclude the presence of a β-lactam resistance. Moreover, the test-system is not able to differentiate between extended activity spectrum β-lactamases (ESBLs), since all react with the chromogenic substrate nitrocefin.

Test procedure

BIOLACTAM is intended for testing of 20 samples plus 2 negative ("Blank") and 2 positive controls. It is recommended to use the assay for a specified number of samples simultaneously. A pipetting scheme is exemplarily illustrated in Figure 1.

Performances of BIOLACTAM

The linearity of the measurement range is ensured approximately between 10 to 80 U penicillinase/mL (corresponding to 0.2 to 1.6 U/sample). The detection limit of nitrocefin is 0.4 μg/mL and the limit of determination 1.9 μg/mL respectively. The reproducibility is indicated by a coefficient of variation <10. The validity of the assay is guaranteed up to a molar absorption coefficient of \( \varepsilon \geq 13000 \) [L x mol\(^{-1}\) x cm\(^{-1}\)] for nitrocefin.

Additional notes

With appropriate storage conditions (-20°C; no repeated freezing) reproducible results were obtained over a period of 3 months using the biological test samples mentioned above.

The validity of the assay was demonstrated with the following microplate reader: Tecan Sunrise (Tecan, Austria) and iMark (BioRad, USA).

Nitrocefin, particularly in solution, is very light sensitive. Observe the instructions of the manufacturer (http://www.oxoid.com/pdf/msds/DE/BR0063.pdf) when handling nitrocefin and disposal of waste containing nitrocefin.

INGESTION OR INHALATION, OR CONTACT WITH THE SKIN AND EYES SHOULD BE AVOIDED!