Read this Instructions For Use carefully before testing.

For research use only

MIZUHO MEDY Co., Ltd.

Reagent for detection of IMP-type metallo-β-lactamase
(β- lactam drug resistance factor)

Quick Chaser® IMP

[Introduction]

Emergence of drug-resistant bacteria associated with large uses of various antimicrobial drugs becomes an issue, although mortality rate caused by bacteria have significantly been decreased recently due to development of various antimicrobial drugs, mostly antibiotics.

β-Lactamases are produced as resistance factor in gram-negative rod including *Enterobacteriaceae, Pseudomonas aeruginosa, Acinetobacter spp.* etc. which are detected in routine laboratory tests. β-Lactamases are enzyme which hydrolyze β-lactam antibiotic and inactivate them. Most of β-lactamases are transmitted by plasmid. Therefore, transmission of resistance factor and easy resistance acquirement are considered possible by contacts between bacteria. Moreover, it means that drug-resistant bacteria could be grown drastically because resistance factors are transmitted regardless of bacterial strains or species. Close attention are required to be paid because drug-resistant bacteria are likely to be spread in facilities or environments. Therefore, there is a compelling need for rapid detection as measure to prevent infection. β -Lactamases are classified into several kinds and metallo- β-lactamase (MBL), extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase are well known. IMP-type MBL are frequently detected from gram-negative rod in Japan. IMP-type MBL are acknowledged as a problem because they degrade not only third generation cephem, but also carbapenem antimicrobial drug like imipenem as last hope.

「Quick Chaser® IMP」is reagent for research use only to detect IMP-type MBL (hereinafter referred to as IMP) in simple steps rapidly and accurately based on the principle of immunochromatography method and can detect IMP producing bacteria, using colonies which were cultured in culture medium as measurement sample.

[General precautions]

1) This product is for research use only. Do not use for other purposes.
2) Extraction reagent solution contains Sodium Azide as preservative. If extraction reagent solution is got into eyes or mouth accidentally, flush with a plenty of water as emergency treatment and see a doctor, if necessary.
3) This product detect IMP-type MBL. There have been reports of existence of many types including NDM type, VIM type, and SIM-1 type and others besides IMP in MBL. Quick Chaser® IMP do not show any cross reactions with NDM-type MBL and VIM-type MBL in the many types. However, there is a possibility of cross reactions with other types of MBL which are similar to IMP-type MBL like SIM-1-type MBL.

[Contents]

1) Test plate − 10 tests
   ・Anti-IMP rat monoclonal antibody
   ・Colloidal gold-labeled anti-IMP rat monoclonal antibody
2) Extraction reagent solution bottle − 15mL / bottle x 1
   Extraction reagent solution is 200mM Tris/HCl buffer with PH7.4 containing 0.09% Sodium azide.
3) Extraction reagent solution vial − 10 vials
4) Swab − 10 pieces
5) Rack (for extraction reagent solution vial) − 1 piece
6) Filter (for extraction reagent solution vial) − 10 pieces
7) Blue cap (for temporary storage of Extraction reagent solution vial) − 10 pieces
8) Name label − 1 sheet

[Intended Use]

Confirmation of IMP-type MBL producing bacteria

[Measurement sample]

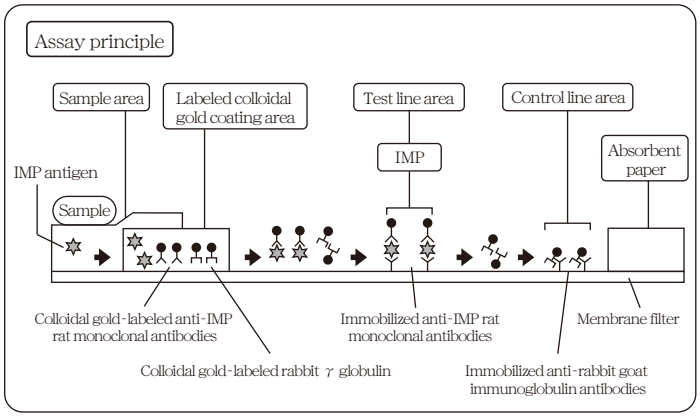
Use colonies of bacteria, isolated and cultured in agar plate media as measurement sample.

[Principle of the test]

「Quick Chaser® IMP」is reagent for research use only to detect IMP-type MBL based on immunochromatography method.

Colloidal gold-labeled anti-IMP rat monoclonal antibodies and colloidal gold-labeled rabbit γ globulin for control line are coated in Labeled colloidal gold coating area in test strip.Moreover, anti-IMP rat monoclonal antibodies are immobilized in the area of Test line. And anti-rabbit goat immunoglobulin antibodies are immobilized in the area of Control line.

According to immunochromatographic principle, when sample are applied onto Sample area in the presence of IMP antigens, they are caught by anti-IMP monoclonal antibodies, immobilized in Test line area to form a sandwich complex, which is made apparent by an " easy to read " visible purple-red line as well. Simultaneouly, colloidal gold-labeled rabbit γ globulin migrate to Control line area, and a red-purple line is made apparent by being caught by anti-rabbit goat immunoglobulin antibodies, immobilized in Control line area, regardless of presence of IMP antigens.

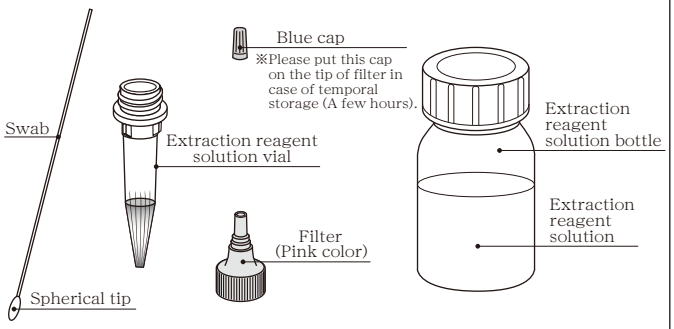


[Procedural precautions]

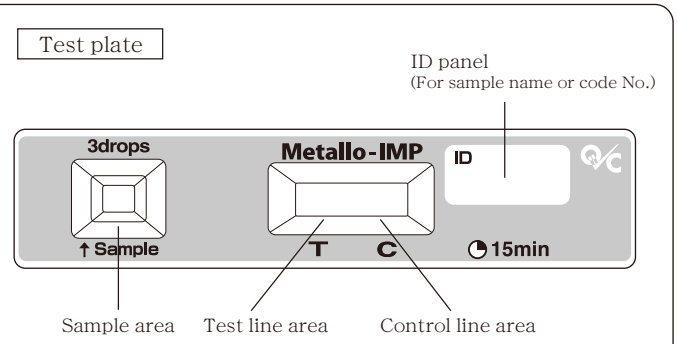
1) Collected colonies should be prepared in accordance with proper method and should be used as soon as possible after preparation.
2) Keep given volume (3 drops) as volume of sample, Proper results might not be obtained in case that the given amount is not kept.
3) Bring test plate and extraction reagent solution to 15 ～ 30℃ prior to use.

[Test procedure]

●Details of extraction reagent solution vial

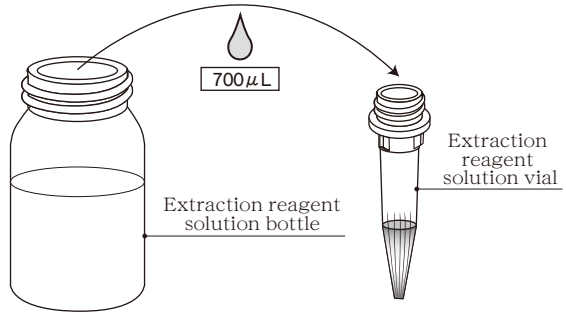


●Test plate



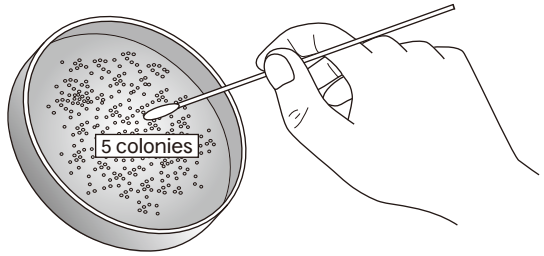
●Preparation of sample

① Dispense 700 μL of extraction reagent solution into extraction reagent solution vial from extraction reagent solution bottle.

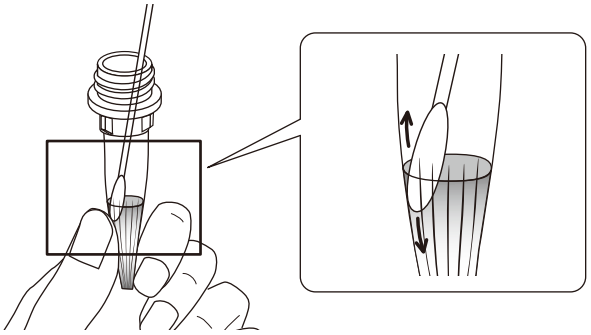


② Collect about 5 colonies grown on cultured media

※ Minimize the amount of attached culture media to avoid the adverse effect on the test at the time of collecting colonies.

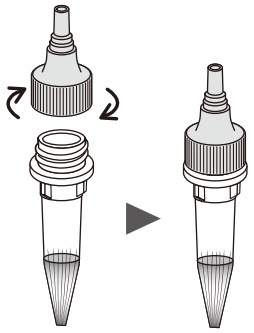


③ Rub the spherical tip of swab with collected colonies on the ragged part in the inside of vial to crush collected colonies. Colonies can be crushed and dissolved in extraction reagent solution efficiently, if they are rubbed near the solution level. Colonies may be suspended and not be able to be crushed well in case of inserting the spherical tip of swab with colonies into the bottom of vial from the beginning.
Pinch the spherical tip with fingers from the outside of vial to suspend colonies and take swab out of vial, squeezing the spherical tip.



④ Install filter (Pink color) and shake the vial right and left gently several times to mix. The sample is ready for use.

※ Extraction efficiency of IMP in bacterial cell are enhanced and clog of filter with bacterial mass are prevented by breaking bacterial mass up.

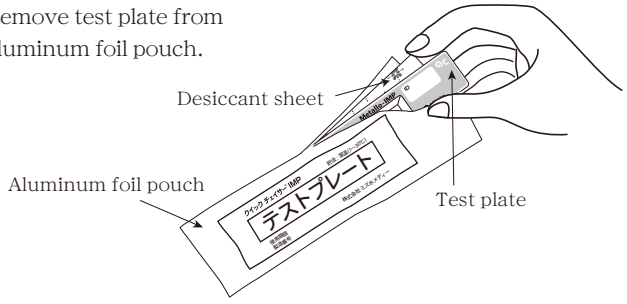


●Test procedure

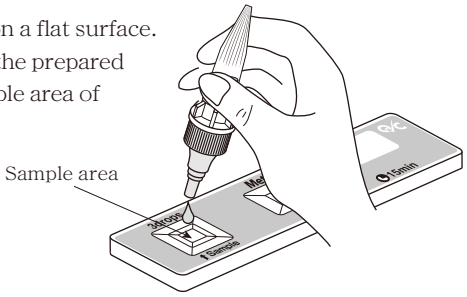
1) Preparation of reagent
Test plate: No prior preparation required.

2) Test procedure

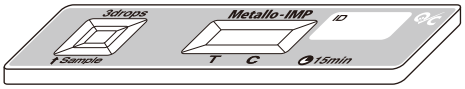
① Remove test plate from aluminum foil pouch.



② Place test plates on a flat surface. Apply 3 drops of the prepared sample onto Sample area of test plate.



③ Leave to react for 15 minutes at 15 ～ 30℃.

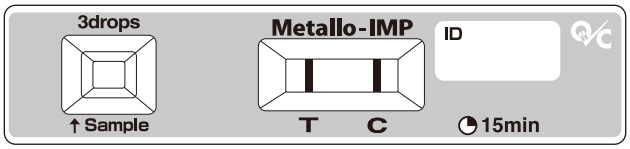


④ Interpret test results visually by lines on test lines area and Control line area.

[Interpretation]

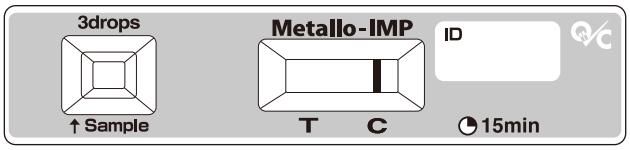
《Positive》

Interpret as positive if test line and control line appear.



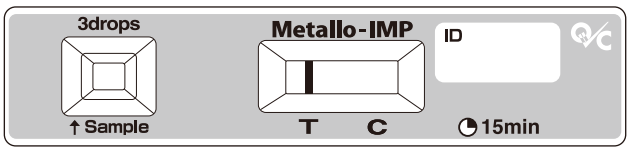
《Negative》

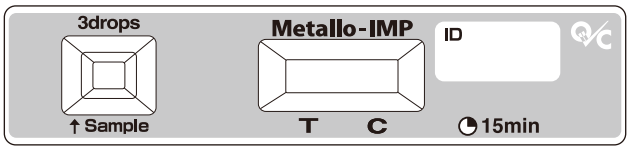
Interpret as negative if only red-purple control line appears.



《Retest》

Control line do not appear regardless of appearances of test line. Inappropriate test procedures such as short sample are conceivable. Recheck test procedure and retest with new reagent.





●Note for interpretation

Interpretation after 15 minutes. Streak line may appear before 15 minutes temporarily. Do not interpret the temporal streak line as appearance of test line.

[Performance characteristics]

		PCR		Total
		+	−	
Quick Chaser® IMP	+	318	0	318
	−	0	60	60
Total		318	60	378

		SMA		Total
		+	−	
Quick Chaser® IMP	+	285	33	318
	−	1	59	60
Total		286	92	378

318 positive strains with *bla*IMP (Gene) showed positive with Quick Chaser® IMP in all cases and 60 negative strains with *bla*IMP (Gene) showed negative with Quick Chaser® IMP in all cases, compared IMP (Protein) test results (by Quick Chaser® IMP) to *bla*IMP (Gene) test results (by PCR) regarding a total of 378 strains: 250 strains of P. aeruginosa and 128 strains of intestinal bacteria. Correlation between Quick Chaser® IMP and PCR was 100%.
Moreover, 285 strains out of 318 positive strains with Quick Chaser® IMP showed positive with SMA method and regarding 33 negative strains with SMA method, the existence of *bla*IMP (Gene) were confirmed with PCR, compared IMP (Protein) test results (by Quick Chaser® IMP) to MBL producing strain screening test results (SMA method) using Sodium mercaptoacetate as inhibitor of MBL.
Furthermore, 59 strains out of 60 positive strains with Quick Chaser® IMP showed negative with SMA method and regarding 1 positive strain with SMA method, the existence of *bla*IMP (Gene) was not confirmed with PCR.

[Precautions for use and handling]

1) Precaution for handling (Prevention of danger)
   ① Handle sample (specimen) as infected substances. It is recommended to wear disposable gloves, goggles and masks etc. for evading the risk of infection at the time of use.
   ② Be careful not to touch sample (specimen) or extraction reagent solution directly to skin or get them into eyes at the time of use.
   ③ If sample (specimen) and/or extraction reagent solution are accidentally got into eyes or mouth, flush with plenty of water as emergency treatment and see a doctor, if necessary.
   ④ Collect sample (specimen) by proficient person or under guidance of proficient person.
   ⑤ Raw material of membrane used for test plate is nitrocellulose. Do not perform test near fire because nitrocellulose is extremely flammable material.
   ⑥ In case sample (specimen) are scattered, wipe them off with alcohol for disinfection.
   ⑦ Do not use blue cap which belong to kit for transportation or preservation because it does not have seal strength.
2) Precaution for use
   ① This product is for research use only. Do not use for treatment, diagnosis and prevention of diseases
   ② Do not mix each kit composition from different lot numbers or different products.
   ③ Do not use reagents beyond expiration date.
   ④ To prevent moisture, do not open the foil pouch until you are ready to perform test.
   ⑤ Do not freeze this product. Store it in accordance with description of instruction for use. Do not use frozen reagents because they could show false results by change of quality.
   ⑥ Do not touch Sample area by hands directly.
   ⑦ Do not touch spherical tip before use.
   ⑧ Test plate, swab, and extraction reagent solution vial (Filter and blue cap are included) are intended for single use only.
   ⑨ Be careful not to splatter the specimen at the time of taking the swab out of vial after preparing specimen.
3) Precaution for waste disposal
   ① Treat used extraction reagent solution vial, used test plate, and used swab as infectious substances by any one of the following methods
      a) Immerse in 2vol% glutaric aldehyde solution for more than 1 hour)
      b) Immerse in Sodium Hypochlorite solution (Valid chlorite concentration 1,000ppm) for more than 1 hour.
      c) Autoclave at 121℃ for more than 20 minutes.
   ② Sodium azide may react with lead or copper plumbing to form explosive metal azide. Use large amount of water to flush discarded test solution.
   ③ Regarding disposal of reagents and utensils etc. , dispose of them in accordance with your local laws and regulations.

[Storage・Expiry]

・Storage: 1 ～ 30℃
・Expiry: 24 months (As indicated on package)

[References]

1) Sekiguchi J. et al.:Antimicrob. Agents Chemother., 49(9), 3734～3742(2005)
2) Sekiguchi J. et al.: J. Clin. Microbiol., 45(3), 979～989(2007)
3) Kouda S. et al.: J. Antimicrob. Chemother., 64(1), 46～51(2009)
4) Kitao T. et al.: Int. J. Antimicrob. Agents., 39(6), 518～521(2012)
5) Kitao T. et al.: J. Microbiol. Methods.,87(3), 330～337(2011)

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