en

Microbiological Assay

REF **MSA04**

60 tests

Positive Blood Cultures Pretreatment Reagent

This is a pretreatment reagent which used for the identification of positive blood culture microorganisms using the AUTOF MS. It is used in conjunction with other clinical and diagnosis procedures as an aid in the early diagnosis of, for example, bloodstream infection.

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		date of manufacture

EC REP

OBELIS S.A.
Bd. Général Wahis, 53
1030 Brussels
Belgium

AUTOBIO DIAGNOSTICS CO., LTD.
No.87 Jingbei Yi Road
National Eco & Tech Development Area
Zhengzhou
China
450016

CE IVD

For any technical assistance please contact us in English at:
Email: customerservice@autobio.com.cn
Contact your local dealers for all product-related questions in your local language

Introduction

In the clinical microbiology laboratories, microbial identification is conventionally done by phenotypic and biochemical analysis mostly using automated systems. They are required time ranging from a few hours to several days depending on microbial species. MALDI-TOF MS technology makes generation of unique mass spectral fingerprints of microorganisms possible, which are mostly a snapshot of ribosomal proteins ideal for an accurate microbial identification at the species level.[1] MALDI-TOF MS can rapidly and accurately identify a wide range of microorganisms at a reasonable cost using only a portion or the entire colony and a drop of matrix solution.[2,3] The ability of MALDI-TOF MS to directly identify bacteria in positive blood cultures is also important for the effective management of bloodstream infections.[4,5]

This is a pretreatment reagent which used for the identification of positive blood culture microorganisms using the AUTOF MS. It is used in conjunction with other clinical and diagnosis procedures as an aid in the early diagnosis of, for example, bloodstream infection.

Measurement Principle

The Positive Blood Cultures Pretreatment Reagent is a reagent which used for pretreatment of blood culture microorganism in the identification of measurand for using with AUTOF MS. The reagent breaks the blood cells from the positive blood culture to separate and enrich microorganisms, as to acquire pathogenic bacteria proteins for analysis using AUTOF MS. Perform the identification in accordance with the instruction of AUTOF MS.

Components

1. Positive Blood Cultures Pretreatment Reagent

4 vials each containing solution with saponin and dimethyl sulfoxide. Reagent provided ready to use.

Σ	60 Tests
Positive Blood Cultures Pretreatment Reagent	3.5 mL *4 vials

Note: The volume of the reagent indicated is the minimum dispensing volume.

Materials Required but not Provided

1. Sample Pretreatment Reagent
2. Centrifuge
3. Micropipette
4. Sterile syringe
5. 1.5mL Centrifuge tube
6. Saline

Assay analyzers on which the reagent can be used

- AUTOF MS

The Positive Blood Cultures Pretreatment Reagent is intended for use on Autof ms.

Warnings and Precautions

1. For professional use only. For *In Vitro* Diagnostic Use.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. When any damage to the protective packaging or any change of dissolubility or usage characteristic is observed, do not use the kit.
4. Avoid cross-contamination when using the micropipette.
5. Blend positive blood bottle by oscillation before collecting culture solution.
6. Avoid vigorously shaking from formation of bubbles.
7. Consider the samples and reagents as potentially infectious material and deal them in accordance with local requirement.
8. Do not use reagent beyond the labeled expiry date. Store the remaining reagent at 2-8 ℃, and be certain the lid is securely sealed.
9. Handle the potentially contaminated materials and wastes safely according to local requirement.
10. Do not smoke, drink, eat or use cosmetics in the working area. Keep the reagents away from fire.
11. Wear protective clothing and disposable gloves when dealing with the Lysate. Wash hands after operations.
12. Conduct the dilution procedure away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
13. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.

Storage

1. Store all components at 2-8 ℃. Do not freeze. When stored as direction, all components are stable until the expiration date.
2. Store and seal the reagent at 2-8 ℃ after opening, under which conditions the stability will be retained for 30 days.

Sample

1. The product applies for the pretreatment of positive blood culture samples.
2. After the positive blood bottle is taken out, the test should be completed within 5 hours; for the blood bottles cannot be tested in time should be stored at 2-8 ℃ and tested within 48 hours.
3. After pretreatment, the sample shall be tested within 2 hours.

Pretreatment Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the AUTOF MS's operation manual.

2. Pretreatment tests
- Collect 1.0mL of blood culture solution form the positive blood bottle into 1.5mL Centrifuge tube by a sterile syringe.
- Add 200μL Positive Blood Cultures Pretreatment Reagent to the centrifuge tube, shake completely and mix well. Then stand at room temperature for 3-5 minutes.
- Centrifuge with 2600 rpm for 10 minutes.
- Remove the supernatant by a micropipette, add 1.0mL of Saline, mix well.
- Centrifuge with 13000 rpm for 2 minutes and remove the supernatant. Add 1.0mL of Saline again and mix well, then centrifuge with 13000 rpm for 2 minutes.
- Remove the supernatant sufficiently. Add 50 μL Lysate 1 of Sample Pretreatment Reagent, mix by pipetting and add isopyknic Lysate 2 of Sample Pretreatment Reagent, mix well.
- Centrifuge with 13000 rpm for 1 minute, add supernatant to the target slide (2 target spots for each bacteria, 1 μL supernatant for each target spot). After dry, overlay the sample spot with 1 μL matrix

solution, and dry the sample spot without obvious water mark.
- Identify the sample with the instrument according to the AUTOF MS's operation manual.

Measurement Results

1. The results of the two target spots are consistent and the score ≥6.0, which means the results are reliable. If one target spot's score is ≥6.0 and another target spot's score is <6.0, then it should subject to the result with the score ≥6.0.
2. If the score of two target spots are <6.0, or the score of two target spots are ≥6.0 but the results of the two target spots are inconsistent, it recommends using other methods of identification.

Limitations of the Procedure

1. The test results cannot be effectively improved by using this kit because of the limitations of the MALDI-TOF MS.
2. If the culture solution contains more than two types of microorganisms, the product cannot be used to complete the accurate identification.
3. The identification rate could not be improved by using strains indistinguishable by 16S and ITS sequencing.

Control Procedure

The recommended control procedure for this assay is to identify the control strains *E.Coli* ATCC®43888, *Staphylococcus aureus* ATCC®BAA-1747 and *Candida albicans* ATCC®10231 separately. Conduct the same operations as in **Pretreatment tests** procedure on the three strains. The result is valid if the identification results should be correct for 1 or more target spots, and *E.Coli, Staphylococcus aureus* and *Candida albicans* are confirmed and the score are ≥6.0.

Performance Characteristics

Use Positive Blood Cultures Pretreatment Reagent for pretreatment of the culture solution of positive blood bottles from 3 control strains (*E.Coli* ATCC®43888, *Staphylococcus aureus* ATCC®BAA-1747 and *Candida albicans* ATCC®10231) follow the instruction of **Pretreatment tests** section. The results were correctly identified to *E. Coli, Staphylococcus aureus* and *Candida albicans,* which the score of at least 1 or more target spots are ≥6.0.

Literature References

1. A. Croxatto, G. Prod'hom, and G. Greub, "Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology," *FEMS Microbiology Reviews*, vol. 36, no. 2, pp. 380-407, 2012.
2. M.A. Claydon, S.N. Davey, V. Edwards-Jines, and D. B. Gordon, "The rapid identification of intact microorganisms using mass spectrometry," *Nature Biotechnology*, vol. 14, no. 11, pp. 1584-1586, 1996.
3. R. D. Holland, J. G. Wilkes, F. Rafii et al., "Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 10, no. 10, pp. 1227-1232, 1996.
4. M. Drancourt, "Detection of microorganisms in blood specimens using matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a review," *Clinical Microbiology and Infection*, vol. 16, no. 11, pp. 1620-1625, 2010.
5. Y. Hoyos-Mallecot, C. Riazzo, C. Miranda-Casas, M. Rojo Martín, J. Gutiérrez-Fernández, and J. Navarro-Marí, "Rapid detection and identification of strains carrying carbapenemasses directly from positive blood culture using MALDI-TOF MS," *Journal of Microbiological Method*, vol. 105, pp. 98-101, 2014.