Summary of Safety and Performance

for Professional Users

This Summary of Safety and Performance (SSP) is intended to provide public access to an up-to-date summary of the main aspects of the safety and performance of the device in accordance with Regulation (EU) 2017/746 and MDCG 2022-9.

The SSP is not intended to replace the Instructions for Use as the main document to ensure the safe use of the device, nor is it intended to provide diagnostic or therapeutic suggestions to intended users.

The following information is intended for professional users.

Document revision：None

Date issued：15/03/2024

Reference number for the SSP：03-00-SSP-000-01，version 1.0

Contents

1. DEVICE IDENTIFICATION AND GENERAL INFORMATION 3
2. INTENDED USE OF THE DEVICE 3
2.1. INTENDED PURPOSE 3
2.2. INDICATION(S) AND TARGET POPULATION(S) 3
2.3. LIMITATIONS AND/OR CONTRA-INDICATIONS 3
3. DEVICE DESCRIPTION 5
3.1. DESCRIPTION OF THE DEVICE, INCLUDING THE CONDITIONS TO USE THE DEVICE 5
3.2. KIT COMPONENTS DESCRIPTION 6
3.3. A REFERENCE TO PREVIOUS GENERATION(S) OR VARIANTS IF SUCH EXISTS, AND A DESCRIPTION OF THE DIFFERENCES 7
3.4. DESCRIPTION OF ANY ACCESSORIES WHICH ARE INTENDED TO BE USED IN COMBINATION WITH THE DEVICE 7
3.5. DESCRIPTION OF ANY OTHER DEVICES AND PRODUCTS WHICH ARE INTENDED TO BE USED IN COMBINATION WITH THE DEVICE 7
4. REFERENCE TO ANY HARMONIZED STANDARDS AND CS APPLIED 7
4.1. RELEVANT STANDARDS AND GUIDELINES 7
4.2. CS 10
5. RISKS AND WARNINGS 10
5.1. RESIDUAL RISKS AND UNDESIRABLE EFFECTS 10
5.2. WARNINGS AND PRECAUTIONS 11
5.3. OTHER RELEVANT ASPECTS OF SAFETY, INCLUDING A SUMMARY OF ANY FIELD SAFETY CORRECTIVE ACTION (FSCA INCLUDING FSN), IF APPLICABLE 12
6. SUMMARY OF PERFORMANCE EVALUATION AND POST-MARKET PERFORMANCE FOLLOW-UP (PMPF) 12
6.1. SUMMARY OF SCIENTIFIC VALIDITY OF THE DEVICE 13
6.2. SUMMARY OF PERFORMANCE DATA FROM THE EQUIVALENT DEVICE, IF APPLICABLE. 17
6.3. SUMMARY OF PERFORMANCE DATA FROM CONDUCTED STUDIES OF THE DEVICE PRIOR TO CE-MARKING 17
6.4. SUMMARY OF PERFORMANCE DATA FROM OTHER SOURCES, IF APPLICABLE 17
6.5. AN OVERALL SUMMARY OF THE PERFORMANCE AND SAFETY 17
6.6. ONGOING OR PLANNED POST-MARKET PERFORMANCE FOLLOW-UP 45
7. METROLOGICAL TRACEABILITY OF ASSIGNED VALUES 45
7.1. EXPLANATION OF THE UNIT OF MEASUREMENT, IF APPLICABLE 45
7.2. IDENTIFICATION OF APPLIED REFERENCE MATERIALS AND/OR REFERENCE MEASUREMENT PROCEDURES OF HIGHER ORDER USED BY THE MANUFACTURER FOR THE CALIBRATION OF THE DEVICE. 45
8. SUGGESTED PROFILE AND TRAINING FOR USERS 46
9. REVISION HISTORY 46

1. Device identification and general information

Device trade name	Dynamiker Aspergillus Galactomannan Assay
Manufacturer's name and address	Dynamiker Biotechnology (Tianjin) Co., Ltd. No.2 Building, Rongzhi Industry Park, No. 3667, Zhongbin Avenue, Sino-Singapore Eco-city, TEDA, Tianjin 300467, China
Manufacturer's single registration number (SRN)	CN-MF-000001133
Basic UDI-DI	69303178MLM03MW
European Medical Device Nomenclature (EMDN) description/text	W01050603
Risk class of device	Class C
whether it is a device for near-patient testing and/or a companion diagnostic	No
Year when the first certificate was issued under Regulation (EU) 2017/746 covering the device	None
Authorized representative's name and SRN	Lotus NL B.V., NL-AR-000000121
NB's name and single identification number	TÜV Rheinland, 0197

2. Intended use of the device

2.1. Intended purpose

The Dynamiker Aspergillus Galactomannan Assay is based on non-automated sandwich Enzyme-Linked Immunosorbent Assay (ELISA). It is used for the qualitative detection of Aspergillus galactomannan antigen in human serum and bronchoalveolar lavage (BAL), offering an aid diagnosis for Invasive Aspergillosis (IA). Patients with long-term neutropenia, hematopoietic stem cell transplantation, solid organ transplantation, glucocorticoid and immunosuppressive therapy, advanced AIDS, chronic granulomatosis and severe pulmonary infection are at high risk of IA infection. This product is intended only for use by professionals in medical institutions.

2.2. Indication(s) and target population(s)

Patients with long-term neutropenia, hematopoietic stem cell transplantation, solid organ transplantation, glucocorticoid and immunosuppressive therapy, advanced AIDS, chronic granulomatosis and severe pulmonary infection are at high risk of IA infection

2.3. Limitations and/or contra-indications

There are no contraindications for the use of this product, and the limitations are as follows:

(1) A negative test from serum and/or BAL samples cannot rule out the diagnosis of Invasive Aspergillosis. Serum samples from patients at risk for Invasive Aspergillosis should be tested twice a week.

(2) The Procedure and the Interpretation of Results must be followed when testing samples for the presence of galactomannan antigen. The user of the kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing, and timing of the incubation steps.

(3) Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. Repeat testing of additional samples should be considered where there is clinical suspicion of Invasive Aspergillosis or procedural error.

(4) Contamination of negative patient specimen wells by positive control/patient specimen wells is possible if the contents of one well spill over into another well due to rough handling of the microplate or poor pipetting technique while adding reagents.

(5) The concomitant use of mold-active anti-fungal therapy in some patients with Invasive Aspergillosis may result in reduced sensitivity with Dynamiker Aspergillus Galactomannan Assay.

(6) The Dynamiker Aspergillus Galactomannan Assay has not been evaluated for use with plasma or other sample types such as urine or CSF.

(7) The performance of the Dynamiker Aspergillus Galactomannan Assay has not been established for manual reading and/ or visual result determination.

(8) Cross-reactivity of BAL fluid samples with Mycoplasma pneumoniae or anaesthetic drugs/lubricants used to numb the neck/throat area for the aspiration process has not been evaluated.

(9) Positive reactions with no clinical signs

The following should be considered with regard to the early galactomannan antigen detection in serum or BAL before the appearance of clinical and/or radiological signs. Positive test results without clinical signs are usually observed and they have been shown to correspond to «true positive» tests in patients for whom Proven or Probable Invasive Aspergillosis diagnosis is established later on.

However, in some particular cases, specific factors should be taken into account when interpreting the test:

a. Positive test results with no clinical signs have been reported, especially in young children. Although some of these cases could be related to real circulation of Aspergillus antigens, most cases can be considered to be false-positives.

b. Galactofuranose has been demonstrated in various foods, particularly cereals, cereal products and cream desserts. Unlike human milk, cow's milk formulas frequently contain high concentrations of galactomannan. Dietary factors must therefore be taken into account in interpretation of the course of antigenemia in young children, and more generally in all

patients with an altered intestinal barrier. Any case of positive antigenemia not accompanied by clinical signs should be interpreted even more cautiously in this population of patients.

c. There have been reports of positive galactomannan test results in patients receiving piperacillin/ tazobactam. There have also been reports of certain lots or batches of piperacillin/ tazobactam that have been found to be positive for galactomannan antigen. Therefore, positive test results in patients receiving piperacillin / tazobactam should be interpreted cautiously and confirmed by other diagnostic methods. Detection of galactomannan has also been reported in some batches of amoxicillin associated with clavulanic acid parenteral preparations. Therefore, semi-synthetic β-lactam treatments should be taken into account when interpreting the test. Nevertheless, as Aspergillus Galactomannan Assay can detect galactomannan antigen well before clinical or radiological signs appear, the occurrence of Invasive Aspergillosis cannot be ruled out. Therefore, patients treated with piperacillin/tazobactam with positive test results should be followed carefully.

d. Positive reactions in the absence of clinical signs may be observed in patients receiving products containing galactomannan, either parenterally or orally (in the presence of an alteration of the intestinal barrier). The presence of galactomannan in these products can often be explained by the use of a fermentation process based on fungal microorganisms. A positive result will not be observed in a patient, however, unless the serum concentration of exogenous galactomannan reaches or exceeds the test’s detection threshold.

e. Thus, if there is a suspicious positive result in the absence of other clinical signs, we recommend investigating the products that the patient is taking and notably their production processes and the origin of the raw materials used.

3. Device description

3.1. Description of the device, including the conditions to use the device

Dynamiker Aspergillus Galactomannan Assay, (hereinafter referred to as the kit), it is composed of Microtiter Strips, Conjugate, Concentrated Washing Solution (20×), Sample Treatment Solution, Substrate Solution, Stopping Solution, Negative Control Serum, Cut-off Control Serum, Positive Control Serum and Plate Sealer.

This product adopts double antibody sandwich enzyme-linked immunosorbent assay, non-automated. Pipette supernatant from pretreated controls, serum or BAL specimens into microwells coated with rabbit anti-galactomannan monoclonal antibody as capture antibody and then incubate. After removing the unbound material by washing, pipette the conjugate as detection reagent into microwells and incubate, the horseradish peroxidase (HRP) conjugated antibody – galactomannan – antibody complex is formed in the presence of galactomannan antigen. Again, after removing the unbound material by washing, the substrate solution is pipetted and incubated, a blue color develops. Then the

stopping solution is added to terminate the color development, where a yellow color develops. The absorbance (optical density) of specimens and controls is determined with a spectrophotometer set at 450 and 620/630 nm wavelength. The color intensity was positively correlated with the content of galactomannan antigen.

The conditions to use the device require room temperature.

3.2. Kit components description

No.	Component	Content	Quantity
R1	Microtiter Strips	12 breakable strips with 8 wells each; coated with *anti*-galactomannan antibodies	1 plate/12×8 wells
R2	Negative control serum	- Human serum negative for galactomannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen	2×1.7 mL
R3	Cut-off control serum	- Human serum containing galactomannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen	2×1.7 mL
R4	Positive control serum	- Human serum containing galactomannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen	2×1.7 mL
R5	Conjugate	Anti-galactomannan antibodies, conjugated with HRP; Preservative: 0.01% thimerosal	1×12 mL
R6	Sample Treatment Solution	EDTA Solution	1×12 mL
R7	Concentrated Washing Solution (20×)	PBS and Tween 20, PH 7.0-7.4 Preservative: 0.05% ProClin300	1×50 mL
R8	Substrate Solution	Tetramethylbenzidine (TMB)	1×12 mL
R9	Stopping Solution	2M H_2SO_4	1×8 mL
M1	Plate Sealer	Adhesive membrane of microtiter plate	3 sheets

Materials needed but not supplied:

(1) Purified water: for the dilution of concentrated washing solution

(2) Absorbent paper

(3) Disposable gloves

(4) Pipette tips (200μL, 300μL, 1000μL)
(5) Pipette (100uL, 1000uL)
(6) Centrifuge (10,000 g)
(7) Polypropylene centrifuge tubes (0.6mL or 1.5mL, sealed and gas-tight)
(8) Vortex mixer
(9) Water bath or Heat block
(10) Incubator
(11) Semi-automatic plate washer (Recommended)

3.3. A reference to previous generation(s) or variants if such exists, and a description of the differences

The specifications of this declaration IVDR product is 96 tests/kit. This product is the first generation product, which has been approved by China Food and Drug Administration on February 14, 2020 and completed self-conformity declaration according to others products under IVDD 98/79/EC on September 18, 2020.10. Compared with the product under the IVDD directive, the intended use and detection principle of the declared product remain unchanged, but the Cutoff of the BAL sample is 0.8, which is consistent with the domestic approved products.

3.4. Description of any accessories which are intended to be used in combination with the device

None

3.5. Description of any other devices and products which are intended to be used in combination with the device

This product should be used in combination with a microplate reader with 450nm (detection wavelength) and 620/630 nm (reference wavelength). Our company recommend the use of Sunrise Tecan and BioTek ELX808IU.

4. Reference to any harmonized standards and CS applied

4.1. Relevant standards and guidelines

Serial No.	Regulation or Standards	Version/ Year
1	EN ISO 13485:2016 Medical devices - Quality management systems - Requirements for regulatory purposes (ISO 13485:2016)	2016
2	EN ISO 14971:2019 Medical devices - Application of risk management to medical devices (ISO 14971:2019)	2019
3	ISO/TR 24971: 2020 Medical device—Guidance on the application of ISO 14971	2020

4	EN ISO 15223-1:2021 Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements (ISO 15223-1:2021)	2021
~~5~~	ISO 18113-1:2022 In Vitro Diagnostic Medical Devices, Information supplied by the manufacturer (labelling) Terms, definitions and general requirements.	2011
~~6~~	ISO 18113-2:2022 In Vitro Diagnostic Medical Devices: information supplied by the manufacturer (labelling), In Vitro Diagnostic Reagents for Professional Use.	2011
7	EN 13612:2002 Performance evaluation of in vitro diagnostic medical devices	2002
8	EN ISO 23640:2015 In vitro diagnostic medical devices - Evaluation of stability of in vitro diagnostic reagents (ISO 23640:2011)	2015
9	IEC 62366-1: 2015 Medical Devices – Application of usability engineering to medical devices	2015
10	ISO:20916-2019 In vitro diagnostic medical devices — Clinical performance studies using specimens from human subjects — Good study practice	2019
11	ASTM D4169-2016 Standard Practice for Performance Testing of Shipping Containers and Systems.	2019
12	ISO14644-1:2015 Cleanrooms and associated controlled environments —Part 1: Classification of air cleanliness by particle concentration	2015
13	EN ISO 14644-2: 2015 Cleanrooms and associated controlled environments Part2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration (ISO 14644-2:2015)	2015
14	(EC) No1272/2008 Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006	2008
15	(EU) 2017/542 COMMISSION REGULATION (EU) 2017/542 of 22 March 2017 amending Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures by adding an Annex on harmonised information relating to emergency health response	2017
16	(EC) No 1907/2006 Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC, and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC, and 2000/21/EC	2006

17	(EU) 2020/878 COMMISSION REGULATION (EU) 2020/878 of 18 June 2020 amending Annex II to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)	2020
18	GHTF SG5 N6 2012 Clinical Evidence for IVD medical devices – Key Definitions and Concepts	2012
19	GHTF/SG5/N7:2012 Scientific Validity Determination and Performance Evaluation - November 2012	2012
20	MEDDEV 2.7.1 rev.4 2016 CLINICAL EVALUATION: A GUIDE FOR MANUFACTURERS AND NOTIFIED BODIES UNDER DIRECTIVES 93/42/EEC and 90/385/EEC	2016
21	CLSI EP17 A2 Protocols for determination of limits of detection and limit of quantitation	A2/2012.6
22	CLSI EP7 A3 Interference testing in clinical chemistry	A3/2018.4
23	CLSI EP37 A1 Supplemental Tables for Interference Testing in Clinical Chemistry	A1/2018.4
24	CLSI EP5 A3 Evaluation of Precision of Quantitative Measurement Procedures	A3/2014.10
25	CLSI EP25 A2 Evaluation of Stability of in Vitro Diagnostic Reagents	A2/2023.4
26	CLSI EP28 A3 Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory	A3/2010.10
27	Guidance on BASIC UDI-DI and changes to UDI-D	MDCG 2018-1 Rev.4
28	Guidance on Article 15 of the medical device regulation (MDR) and in vitro diagnostic device regulation (IVDR) on a ‘person responsible for regulatory compliance’ (PRRC)	MDCG 2019-07 Rev.1
29	Guidance on sampling of MDR Class IIa / Class IIb and IVDR Class B / Class C devices for the assessment of the technical documentation	MDCG 2019-13
30	Clinical Evaluation - Equivalence A guide for manufacturers and notified bodies	MDCG 2020-5
31	Post-market clinical follow-up (PMCF) Plan Template A guide for manufacturers and notified bodies	MDCG 2020-7
32	Post-market clinical follow-up (PMCF) Evaluation Report Template A guide for manufacturers and notified bodies	MDCG 2020-8
33	Clinical evaluation assessment report template	MDCG 2020-13

34	Guidance on Classification Rules for in vitro Diagnostic Medical Devices under Regulation (EU) 2017/746	MDCG 2020-16 rev.2
35	Performance study application/notification documents under Regulation (EU) 2017/746	MDCG 2020-19
36	FAQ on the European Medical Device Nomenclature (EMDN)	MDCG 2021-12
37	Explanatory note on IVDR codes	MDCG 2021-14
38	Questions and Answers on Articles 13 & 14 of Regulation (EU) 2017/745 and Regulation (EU) 2017/746	MDCG 2021-27 Rev.1
39	Guidance on general principles of clinical evidence for In Vitro Diagnostic medical devices (IVDs)	MDCG 2022-2
40	Guidance on significant changes regarding the transitional provision under Article 110(3) of the IVDR	MDCG 2022-6
41	Questions and Answers on the Unique Device Identification system under Regulation (EU) 2017/745 and Regulation (EU) 2017/746	MDCG 2022-7
42	Regulation (EU) 2017/746 - application of IVDR requirements to 'legacy devices' and to devices placed on the market prior to 26 May 2022 in accordance with Directive 98/79/EC	MDCG 2022-8
43	Summary of safety and performance Template	MDCG 2022-9
44	IVDR – national language requirements for manufacturers (January 2024)	/

4.2. CS

Not applicable, only common specifications for class D products are currently available.

5. Risks and warnings

5.1. Residual risks and undesirable effects

A total of 40 risk points were identified, of which 14 risks were considered to be "acceptable risks", 26 risks were considered to be "reasonably feasible risks to reduce", and after risk control measures were taken, they were considered to be "acceptable risks" and no unacceptable risks were found.

Risk analysis covers all potential risks and hazards related to product design, production, user use and safety. A total of 40 risks were identified, 8 related to product design, 18 related to product production, and 14 related to user use and safety. These risks may have an impact on the accuracy of

product test results, the effectiveness of reagents, the integrity and accuracy of reagent operation, environmental pollution, the quality of production products, the timeliness of production, and the rational use of resources. Therefore, these risks are considered to be within the scope of this risk management document. All risks are considered "acceptable risks" after implementation of risk control measures, including but not limited to training that takes into account the inherent safety of product design and manufacturing, safety information in instructions or labels, and, where appropriate, user training. Based on this review, it has been determined that any risks recorded with this product are properly controlled and have been minimized to the extent possible without affecting the benefit/risk ratio.

5.2. Warnings and precautions

All of the following warnings and precautions are included in the product instructions and can be traced back to the Risk Evaluation and Risk Control Measures Evaluation Form in the Risk Management report.

5.2.1. Warnings

(1) For in vitro diagnostic use.

(2) For professional use only.

(3) In the event of any serious adverse reaction involving the device during use, the user should report it to the manufacturer and to the competent authority in the Member State where the user is located.

(4) Do not pipette by mouth.

(5) Wear protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) and handle the kit reagents and patient samples with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.

(6) Avoid splashing samples or solutions.

(7) Biological spills not containing acid should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), 70% ethanol. Materials used to wipe up spills may require biohazardous waste disposal.
CAUTION: Do not place solutions containing bleach in the autoclave.

(8) Spills containing acid should be appropriately absorbed (wiped up) or neutralized with sodium bicarbonate, and the area rinsed and wiped dry; if it contained biohazardous material, wipe the area with one of the chemical disinfectants.

(9) Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous wastes must be handled and discarded in accordance with all local, regional and national regulations.

5.2.2. Precautions

(1) Use of this test kit with samples other than human serum and BAL fluid is not recommended.

(2) Frozen serum or BAL samples stored in unknown conditions may give inaccurate results due to contamination with fungus and/or bacterial.

(3) Do not use kit or any kit reagents after the stated expiration date.

(4) Do not mix reagents from other kits that have different lot numbers.

(5) Bring all reagents to room temperature for at least 30 minutes before use.

(6) Mix thoroughly every reagent before use.

(7) Mix thoroughly the Concentrated Washing Solution (R7) before preparing the Working Washing Solution, exercising care to avoid microbial contamination, if crystallization occurs in the concentrated washing solution, please dissolve it before use.

(8) Do not conduct the test in the presence of reactive vapors (acids, alkalis, aldehydes) or dust, which could affect the enzymatic activity of the Conjugate.

(9) For manual pipetting of controls and specimens, use individual pipette tips to prevent carryover of samples.

(10) To ensure adequate washing of the wells, comply with the recommended number of wash cycles and ensure that all wells are completely filled and soak 40 seconds, then completely emptied. Washing should not be performed manually with a squeeze bottle.

(11) Do not allow the microplate to dry between the end of the wash cycle and addition of reagents.

(12) Do not use the same container for the Conjugate and Substrate Solution.

(13) Do not allow Conjugate or Substrate Solution to come into contact with metal or metallic ions.

(14) Avoid exposing the Substrate Solution to strong light during storage or incubation. Do not allow the substrate solutions to come into contact with an oxidizing agent.

(15) Avoid contact of the Stopping Solution with any oxidizing agent. Do not allow the Stopping Solution to come into contact with metal or metallic ions.

(16) Use clean, dust-free materials (tubes, tips, containers, etc.) to minimize the possibility of contamination with Aspergillus spores from the environment. Because galactomannan is heat-stable, sterilization of material used does not guarantee the absence of contaminating antigen. Pyrogen-free materials are optimal, but standard material can be used with adequate precautions.

(17) Limit exposure of solutions (sera, BAL fluid, Sample Treatment Solution, Conjugate) or open containers (plates, tubes, pipettes) to the air.

(18) Do not pour any unused Conjugate back into the original container.

(19) The Substrate TMB Solution must be colorless. The appearance of a blue color indicates the reagent is contaminated and should not be used.

5.3. Other relevant aspects of safety, including a summary of any field safety corrective action (FSCA including FSN), if applicable

There are no other relevant security aspects and no FSCA or FSN associated with the product.

6. Summary of performance evaluation and post-market performance follow-up

(PMPF)

6.1. Summary of scientific validity of the device

This study evaluates the scientific validity based on the following sources: (1) consensus expert opinions from relevant professional associations; (2) peer-reviewed literature; (3) results from clinical performance studies.

6.1.1. Consensus expert opinions from relevant professional associations

The EORTC/MSGERC consensus criteria were established in 2002 to more homogenously define cases of IA in clinical research, and have been revised in 2008 and 2019, respectively. These definitions have been adopted widely by regulatory agencies. Cases of IA were categorized as proven, probable or possible. The divisions are based on host factor criteria, clinical criteria and microbiological criteria.

Biomarkers have potential to detect infection before development of overt disease, allowing treatment to be initiated at an earlier stage. A positive galactomannan test, whichever fluid it is applied on, is a microbiological criterion in 2002, 2008 and 2019 versions.

The optimal cut-off value of the optical density index of the galactomannan antigen assays (GM) for diagnosing IA is a disputed topic. Originally, the serum cut-off for positivity was set at ≥1.5; however, the 2008 EORCT/MSG guideline advised to lower it to ≥0.5 after a review by the FDA. In the updated EORCT/MSG report of 2020, the cut-off was increased to a cut-off value of ≥1.0 for both or when combined lower cut-offs, ≥0.7 for serum and ≥0.8 for BAL, are advised.

6.1.2. Peer-reviewed literature

Peer-reviewed literature was classified into meta-analysis literature dataset, clinical research literature dataset and review and comment literature dataset.

6.1.2.1 Meta-analysis literature dataset

Two meta-analysis studies on serum galactomannan have been included.

Husain 2007 focused mainly on haematological patients and included 27 studies. At a cut-off level of 0.5, the sensitivity was found to be 0.79 and the specificity 0.85. At a cut-off level of 1.0, the sensitivity was found to be 0.65 and the specificity 0.94. At a cut-off of 1.5 sensitivity dropped to 0.48 and specificity rose to 0.95.

Leeflang 2015 focused specifically on immunocompromised patients and included 50 studies. At a cut-off level of 0.5, the sensitivity was found to be 0.82 and the specificity 0.81. At a cut-off level of 1.0, the sensitivity was found to be 0.72 and the specificity 0.88. At a cut-off of 1.5 sensitivity dropped to 0.61 and specificity rose to 0.97.

6.1.2.2 Review and comment literature dataset

Twenty-six review and comment studies have been included. These studies mainly focus on the following issues: advances in GM detection for diagnosis of IA, clinical performance of BAL samples, clinical performance of pediatric patients, factors affecting diagnostic accuracy, and false negative and false positive reactivity. The summary of scientific validity analysis is as follows.

The optimal cut-off value of the optical density index of the galactomannan antigen assays (GM) for diagnosing IA is a disputed topic. The cut-off level of 1·5 ng/L initially recommended by Bio-Rad and used in many early studies has been progressively revised downwards; a cut-off of 0·5 ng/mL was then accepted by the US Food and Drug Administration (FDA). Currently, the most common cutoff values for serum of BAL are 0.5, 1.0, or 1.5. Testing two consecutive samples or a screening strategy for two to three times may have more reliable results.

GM test is used mainly in immunosuppressed or neutropenic patients. There was high degree of heterogeneity in sensitivity or specificity among these studies. Large heterogeneity of results related to the differences in patient populations and study designs was reported among these studies. It is shown that GM studies consistently indicate a better performance of BAL GM than serum GM. The sensitivity and specificity of GM testing in pediatric patients may be lower than in adults.

The main factors that affect diagnostic accuracy are as follows:

(1) Population, including demographic features, disease severity, disease prevalence, selection of participants.

(2) Test protocol, including test execution, test technology, treatment paradox, disease progression bias.

(3) Reference standard and verification procedure, including inappropriate reference standard, differential verification bias, partial verification bias.

(4) Interpretation, including incorporation bias, observer variability.

(5) Analysis, including handling of indeterminate results, arbitrary choice of threshold value.

According to relevant reports, the main factors that affect false positive GM result:

(1) Patients receiving some beta-lactam antibiotics, especially piperacillin/tazobactam or amoxicillin/clavulanic acid.

(2) Galactomannan is a complex sugar found in many food products. If the intestinal mucosal barrier is damaged, it occurs the passage of the GM from the food into the bloodstream.

(3) Galactomannan is also found in other fungi. Other moulds including Penicillium and Paecilomyces and Some non-Aspergillus molds such as Penicillium marneffei were reported to cross-react with Aspergillus GM epitopes.

(4) Lipoteichoic acid from the bacterium Bifidobacterium may cross-react with the assay

The main factors that affect false negative GM results are:

(1) A lower clinical utility and false negative serum GM test is possible among patients treated with antifungal prophylaxis affecting Aspergillus spp.

(2) GM testing in children may have false negative results in certain pediatric patients at high risk for IA such as those with CGD.

Numerous biological (such as site of infection, Aspergillus species, presence of neutropenia) and epidemiological factors (patient population, definition of IFD, prevalence of IFD, cut-off of positivity) influence the performance of GM for the diagnosis of IA.

6.1.2.3 Clinical research literature dataset

Forty-eight clinical research studies have been included. These studies mainly focus on the following issues: clinical performance of serum GM detection, clinical performance of GM detection in broncho-alveolar lavage fluid, clinical performance of GM detection in pediatric patients and false positive in GM results.

The sensitivity and specificity data of 27 articles discussing clinical performance of GM detection in serum are extractable. In total, 26 studies were included with a patient size of 4505 patients including 948 proven or probable IA. 1 studies were included with a sample size of 28 samples, which did not mention the patient size. The performance evaluation results of multiple cutoff value were reported in 5 studies (Hadrich 2012, Zhang 2015, Aerts 2022, Maertens 2004, Kawazu 2004). Literature on the use of serum GM for diagnosis of IA shows wide variation in diagnostic Sensitivity for these 26 studies ranged between 23% and 100% and specificity range between 65.7% and 100%.

Thirteen studies discussed Clinical performance of GM detection in broncho-alveolar lavage fluid. In total, 11 studies were included with a patient size of 1110 patients including 400 proven or probable IA. 2 studies were included with a sample size of 178 samples, which did not mention the patient size. One of these 2 studies did not mention the number of proven or probable samples. The performance evaluation results of multiple cutoff value were reported in 4 studies (Maertens 2009, D'Haese 2012, Zhang 2015, Buil 2014). Literature on the use of BAL GM for diagnosis of IA shows wide variation in diagnostic Sensitivity for these 11 studies ranged between 57% and 100% and specificity range between 54.5% and 100%.

There were many potential sources of heterogeneity for the results of GM detection in serum and BAL. These include study design, differences in populations, sampling, Aspergillus species, site of the infection and threshold for positivity. Several studies included small number of patients with the target condition.

Four studies discussed clinical performance of GM detection in pediatric patients. One study discussed the clinical performance of BAL GM detection in pediatric patients. Three studied discussed the clinical performance of serum GM detection in pediatric patients. The populations and the results of the studies were heterogeneous. In these three studies that tested GM in serum, two studies used 0.5 as the cutoff value, 1 study suggested that the optimal cutoff value is 1.5. These studies applied to different populations, and have different GM detection ways. This studied have certain limitations, such as the number of proven cases was too low, the effect of confounders giving false positive GM antigen test results like use of antibiotics and dietary contamination which were not excluded, etc..

Six studies discussed False positive in GM testing. Of these, four studies discussed false positive galactomannan due to piperacillin-tazobactam, one study discussed false positive galactomannan due to amoxicillin-clavulanate, one study discussed false positive galactomannan due to total parenteral nutrition products.

For 4 studies discussed false positive galactomannan due to piperacillin-tazobactam, Gerlinger 2020 suggested that the association between false galactomannan positivity and piperacillin-tazobactam is not longer systematic, but can still prevail depending on the manufacturers; Alhambra 2007 suggested

that there is a significant association was found between false positive GM and the administration of piperacillin-tazobactam; Mikulska suggested that currently available brand piperacillin/tazobactam preparations seem no longer responsible for false-positive GM results; Vergidis 2004 suggested that piperacillin-tazobactam formulations commonly used in the United States appear to be a rare cause for false-positive GM results.

For 1 study discussed false positive galactomannan due to amoxicillin-clavulanate, Chong 2023 demonstrated that the glucose component of the TPN products contained a high level of GM antigen, which caused false-positive GM test results.

For 1 study discussed false positive galactomannan due to total parenteral nutrition products, Zandijk 2008 demonstrated treatment with amoxicillin-clavulanate can give a false-positive GM test result.

6.1.3 Clinical research literature dataset

The comparison test carried out in China (this product vs Bio Rad's Platelia™ Aspergillus EIA reagent kit) showed that when cutoff was 0.5, the positive agreement rate of 1024 serum samples was 93.60%, the negative agreement rate was 96.12%, and the total agreement rate was 95.31%. The results of foreign laboratory evaluation showed that the positive agreement rate of this product with Bio Rad's Platelia™ Aspergillus EIA reagent kit for serum and BALF samples was more than 90%, and the negative agreement rate was more than 96%. The above results show that this product has good clinical detection consistency with the marketed recognized reagents. In the foreign evaluation data, compared with the clinical reference standard, the sensitivity and specificity of GM serum test in the proven group and probable group were 79.3% and 80.5% at cutoff 0.5, and the sensitivity of GM serum test in the peer-reviewed literature ranged from 23% to 100%, which indicated that there was still a certain false positive rate in GM serum test. When the test result was positive, repeat test should be recommended and combined with other clinical evidence for final diagnosis.

6.1.4 Conclusions

Aspergillus galactomannan (GM) antigen detection in serum by the Bio-Rad Platelia sandwich enzyme immunoassay (EIA) has been studied extensively and has gained widespread acceptance as a sensitive method for diagnosing IA. A positive galactomannan test, whichever fluid it is applied on, is a microbiological criterion in 2002, 2008 and 2019 versions of EORTC/MSGERC consensus.

The optimal cut-off value of the optical density index of the GM for diagnosing IA is a disputed topic. The results of the serum and BAL GM detection for adults and children have high heterogeneity. There were many potential sources of heterogeneity. These include study design, differences in populations, sampling, Aspergillus species, site of the infection and threshold for positivity. Several studies included small number of patients with the target condition. Meanwhile, some factors may cause false positive or false negative results.

However, in these articles, it is shown that in certain expected populations, selecting specific cutoff value results in higher accuracy of serum and BAL GM detection. Therefore, the scientific validity of serum and BAL GM detection has been confirmed.

6.2. Summary of performance data from the equivalent device, if applicable.

Appendix IX, Point 4.5, and Appendix X, point 3(d) refer to clinical evidence based in part or in whole on data from published devices that are similar or identical to the devices being evaluated. The clinical evidence for this product does not use this method.

6.3. Summary of performance data from conducted studies of the device prior to CE-marking

The product in the scope of this report is a legacy product and was CE mark under Directive 98/97/EC, therefore, no performance data available prior to CE- marking.

6.4. Summary of performance data from other sources, if applicable

Not applicable. The performance data of the product was based on our analytical performance data (see section 6.5)

6.5. An overall summary of the performance and safety

6.5.1. Analytical Performance Summary

The analytical sensitivity, analytical specificity, precision, HooK effect, positive judgment value, kit stability and sample stability of this product were studied

6.5.1.1 Analytical sensitivity

Experimental design	Acceptable criteria	Conclusion
Determination of the minimum detection limit: Aspergillus galactomannan was diluted with negative serum matrix to 0.340, 0.375, 0.413 and 0.454 ng/mL, and Aspergillus galactomannan was diluted with mixed negative alveolar lavage fluid to 0.680, 0.748, 0.823 and 0.905 ng/mL, respectively. 20 parallel samples were made for each sample, and the minimum detection limit was tested using 3 batch kits. Verification of the minimum detection limits: Samples with 80% and 120% minimum detection limits were obtained by diluting Aspergillus galactomannan with negative serum matrix, and 20 parallel samples were made for each sample	Determination of the minimum detection limit: positive coincidence rate ≥95%. Verification of the minimum detection limit: the probability of detection negative for samples with 80% minimum detection limit concentration is ≥95%, and the probability of detection positive for samples with 120% minimum detection limit concentration is ≥95%.	The minimum detection limit of serum samples was 0.375 ng/mL. The minimum concentration limit of BAL samples was 0.748 ng/mL.

6.5.1.2 Analytical specificity

Experimental type	Experimental design	Acceptable criteria	Conclusion

Sample with possible cross-reaction	Serum samples and alveolar lavage fluid samples of aspergillus galactomannan negative and positive candida, cryptococcus, Streptococcus pneumoniae, mycoplasma pneumoniae and Bacillus tuberculosis were collected in 10 cases each. Culture medium of yeast, coccidioides and hepatitis C virus were added to negative serum and negative alveolar lavage fluid, 10 parts each. The above samples were tested using 3 batch kits.	Negative coincidence rate ≥100%	Candida, cryptococcus, Streptococcus pneumoniae, Mycoplasma pneumoniae, Mycobacterium tuberculosis, yeast, coccidioides and hepatitis C virus were not cross-reactive with this kit.
Sample with possible interference substance	Hemoglobin was added to the samples prepared by the enterprise to the final concentration of 0mg/mL, 1mg/mL, 3mg/mL, 5mg/mL and 7mg/mL, respectively. Three batch kits were used to detect potentially interference substances	If the negative preparation sample has false positive or the positive preparation sample I value is significantly reduced, it is considered that the concentration of hemoglobin has interfered with the detection result.	When the hemoglobin concentration in the sample is less than 7mg/mL, there is no significant interference with the detection results.
	Unconjugated bilirubin was added to the samples prepared by the enterprise to the final concentration of 0mg/L, 30mg/L, 75mg/L, 150mg/L, 200mg/L, 300 mg/L, 400 mg/L. Three batch kits were used to detect potentially interference substances	If the negative preparation sample has false positive or the positive preparation sample I value is significantly reduced, it is considered that the concentration of bilirubin has interfered with the detection result.	When the concentration of unconjugated bilirubin in the sample is less than 400mg/L, there is no significant interference with the detection results.

	Triglyceride was added to the samples prepared by the enterprise to the final concentration of 0mmol/L, 0.75mmol/L, 1.5mmol/L, 3mmol/L, 4.5mmol/L, 6 mmol/L, 7.5mmol/L 37 mmol/L, 40 mmol/L. Three batch kits were used to detect potentially interference substances	If the negative preparation sample has false positive or the positive preparation sample I value is significantly reduced, it is considered that the concentration of hemoglobin has interfered with the detection result.	When the triglyceride in the sample is less than 40 mmoL/L, it has no significant influence on the detection result.
	The positive samples of rheumatoid factor, anti-nuclear antibody and anti-double-stranded DNA antibody were detected by 3 batches of kits	Negative coincidence rate ≥100%	Rheumatoid factor, anti-nuclear antibody and anti-double-stranded DNA antibody had no influence on the detection results.
	Drug A (Voriconazole), drug B (amphotericin B) and drug C (carpofungin) were added to the samples prepared by enterprise to the final concentration of A (40μg/mL), B (1mg/mL) and C (0.27 mg/mL). Three batch kits were used to detect potentially interference substances	If the negative preparation sample has false positive or the positive preparation sample I value is significantly reduced, it is considered that the concentration of drug has interfered with the detection result.	Common drugs A (Voriconazole, 40 μg/mL), B (amphotericin B, 1 mg/mL) and C (carpofungin, 0.27 mg/mL) had no effect on the detection results.

6.5.1.3 Precision

Experimental design	Acceptable criteria	Conclusion

5 serum samples (1 negative sample, 1 high negative sample, 1 critical positive sample, 1 medium positive sample, 1 strong positive sample) and 5 BAL samples (1 negative sample, 1 high negative sample, 1 critical positive sample, 1 medium positive sample, 1 strong positive sample) collected in 3 laboratories were tested respectively. 3 batches of kits were used by 1 operator in each laboratory, and 5 replicates were performed per sample per day for a total of 5 days. Detection index: Repeatability, indoor precision and inter-batch precision of medium positive and strong positive samples; The positive detection rate of critical positive samples; Negative detection rate for negative/high-value negative samples	1. Medium positive/strong positive samples: repeatability ≤10%, indoor precision ≤10%, inter-batch precision ≤15% 2. Critical positive sample: critical positive detection rate ≥95% 3. Negative/high-value negative samples: the negative detection rate should be 100%	1. Serum samples: Repeatability and precision in laboratory were less than 10%, and precision between batches was less than 15%; The positive detection rate of critical positive serum samples was ≥95%. The negative detection rate of both negative and high-value negative serum samples was 100%. 2.BAL sample: Repeatability, indoor precision is less than 10%, batch precision is less than 15%; The positive detection rate of critical positive serum samples was ≥95%. The negative detection rate of both negative and high-value negative serum samples was 100%.

6.5.1.4 HooK effect

Experimental design	Acceptable criteria	Conclusion
The 100 μg/mL galactomannan antigen was diluted into different concentrations of 0.1 ng/mL, 1ng/mL, 10ng/mL, 100ng/mL, 1000ng/mL and 10000 ng/mL with negative serum matrix and mixed negative alveolar lavage fluid, respectively. HOOK effect indicators were detected using 3 batch kits	If there is no HOOK effect, 10000 ng/mL is positive	There was no HOOK effect when the concentration of galactomannan antigen was 10000 ng/mL

6.5.1.5 Cutoff

Experimental design	Acceptable criteria	Conclusion

Determination of positive judgment value: The Aspergillus galactomannan detection kit (enzyme-linked immunoassay) produced by our company was used to determine 130 healthy samples (serum/alveolar lavage fluid) in strict accordance with the detection methods described in the instructions, and the detection results of 130 non-aspergillus infection samples were sorted and analyzed, and 97.5% sub-sites were taken as positive judgment value. Validation of positive judgment values: The detection results of 65 healthy samples (serum/alveolar lavage fluid) and 65 samples of patients excluded from Aspergillus infection (serum/alveolar lavage fluid) were respectively detected for sequencing analysis, and 97.5% sub-sites were taken as the validation critical value.	If the relative deviation between the verified critical value and the confirmed critical value is less than 15%, it is considered that the test results of the samples with non-aspergillus infection are consistent with those of the patients with aspergillus infection excluded, and the verification is passed	The positive judgement value of serum was 0.50 and that of alveolar lavage fluid was 0.80.

6.5.1.6 Stability

Experimental type	**Experimental design**	**Acceptable criteria**	**Conclusion**
Sample stability at 2-8 ℃	The collected 10 positive serum samples, 10 negative serum samples, 10 positive BAL samples and 10 negative BAL samples were placed at 2~8℃, and removed at 0h, 12h, 24h, 36h, 48h, 60h, 72h and 78h respectively. A batch kit was used to detect the negative and positive samples.	The negative coincidence rate was 100% The positive coincidence rate was 100%	Serum samples were stable for 72h at 2-8 ℃. The stability of the alveolar lavage fluid samples was 72h at 2-8 ℃.
Sample stability at -20 ℃	The collected 10 positive serum samples, 10 negative serum samples, 10 positive BAL samples and 10 negative BAL samples were placed in the refrigerator at -20 ℃ and removed at 0, 3, 6, 9, 12, 15, 18 and 19 months, respectively. A batch kit was used to test the negative and positive samples.	The negative coincidence rate was 100% The positive coincidence rate was 100%	Serum samples were stable for 18 months at -20℃. The stability of the alveolar lavage fluid samples was 18 months at -20℃
Sample freeze-thaw stability	The collected 10 positive serum samples, 10 negative serum samples, 10 positive BAL samples and 10 negative BAL samples were subpackaged and placed in the refrigerator at -20℃, and were taken out after repeated freezing and thawing 0, 1, 2, 3, 4 and 5 times	The negative coincidence rate was 100% The positive coincidence rate was 100%.	The stability of serum samples was frozen and thawed 4 times at -20℃. The stability of the alveolar lavage fluid samples was frozen and

	respectively. A batch kit was used to detect the negative and positive samples.		thawed for 4 times at -20 ℃
Real-time stability	The product was stored at 2-8 ℃, and the kit was taken out at 0, 3, 6, 9, 12, 14 months, respectively, to determine the negative and positive of the samples prepared by the enterprise (negative reference products, positive reference products, sensitivity reference products and repetitive reference products).	Positive coincidence rate ≥95%, negative coincidence rate ≥95%, sensitivity coincidence rate ≥95%, repeatability CV < 10%.	The real-time stability of the kit was 12 months when it was stored at 2~8 ℃.
In-use stability	After each component of the product is opened, it is stored at 2~8℃ for 0, 7, 14, 21, 28, 42 and 60 days, respectively, and the positive and negative of the samples prepared by the enterprise (negative reference products, positive reference products, sensitivity reference products and repetitive reference products) are determined.	Positive coincidence rate ≥95%, negative coincidence rate ≥95%, sensitivity coincidence rate ≥95%, repeatability CV < 10%.	When the kit was stored at 2~8 ℃, the opening stability of the product was 8 weeks.
Transportation stability	The product was placed in a foam box with an ice pack, transported from Tianjin to Urumqi and then back to Tianjin, arrived in Tianjin and then transported to Sanya, arrived in Sanya and then shipped back to Tianjin (a total of 12,000 kilometers), the transportation time was January 2022, a total of 22 days. After returning to the company, it is normally stored in the cold storage at 2~8 ℃. The samples were taken out at 0 months, 3 months, 6 months, 9 months, 12 months and 14 months after the production date, and the positive and negative of the samples prepared by the enterprise (negative reference products, positive reference products, sensitivity reference products and repetitive reference products) were determined.	Positive coincidence rate ≥95%, negative coincidence rate ≥95%, sensitivity coincidence rate ≥95%, repeatability CV < 10%.	When the kit is stored at 2~8 ℃, the transport stability of the product is 12 months.
Accelerated stability	The three batches of kits were stored at 37℃ for 0, 6, 12, 18, 21, 24 and 28 days, respectively, to determine the negative and positive of the samples prepared by the	Positive coincidence rate ≥95%, negative coincidence rate	The kit was accelerated at 37℃ for 28 days with good performance.

	enterprise (negative reference products, positive reference products, sensitivity reference products and repetitive reference products), and evaluate whether each performance index met the acceptance criteria. The opening stability of the kit was determined.	≥95%, sensitivity coincidence rate ≥95%, repeatability CV < 10%.	

6.5.2. Clinical Performance Summary

6.5.2.1 Study on clinical performance of serum in China

(1) Test method

Comparative clinical research method was adopted in this experiment, that is, the assessment reagent (this product) and the PLATELIA™ ASPERGILLUS Ag produced by Bio-Rad Company, which has been listed in China, were tested on the same sample, and the consistency of the results was compared and analyzed. The positive coincidence rate, negative coincidence rate, total coincidence rate and Kappa value of the assessment reagent and the comparison reagent were evaluated.

The positive coincidence rate, negative coincidence rate and overall coincidence rate of the assessment reagent were statistically analyzed by using the 2 x2 table method, and Kappa consistency analysis was carried out. Kappa value ≥0.75 indicates that the results of the two kits are highly consistent. 0.4≤Kappa value < 0.75 indicates that the detection results of the two kits are consistent, and the positive and negative coincidence rates need to be compared and statistically analyzed. If Kappa value < 0.4, the results of the two kits were inconsistent.

This clinical trial was conducted in no less than 2 clinical trial institutions, with a total sample size of no less than 200 cases; The number of positive samples shall not be less than 30% of the total sample size, and the rest shall be negative samples. Among the negative samples, there shall be no less than 10 positive samples of other pathogens that are prone to cross-reaction (that is, samples of patients who are positive for candida, pneumonia, tuberculosis and other pathogens); The sample size of each clinical trial facility should be distributed as evenly as possible. The selected samples can be the remaining frozen or fresh serum samples of the subjects' daily testing, including prospective samples and retrospective samples.

(2) Test results

In this clinical trial, a total of 1024 serum samples were screened in Jiangxi Provincial People's Hospital, Shandong Chest Hospital, Tianjin Medical University General Hospital, and Hematological Disease Hospital of Chinese Academy of Medical Sciences, 1024 were enrolled, 0 were excluded, and 0 were shed, and a total of 1024 were completed. Among the 1024 samples, 626 were male, accounting for 61.13%. 398 cases were female, accounting for 38.87%; There were 804 fresh samples, accounting for 78.52%; 220 cases were frozen, accounting for 21.48%. The enrolled samples of this clinical trial included 28 confirmed samples, 278 clinically diagnosed samples, 235 proposed samples, and 483 control samples (321 of which were expected population).

A total of 1024 serum samples were tested in this clinical trial, of which 307 were positive with both assessment reagent and comparison reagent, 669 were negative with both assessment reagent and comparison reagent, 48 were inconsistent. Through the four-cell table analysis of 1024 samples, it can be seen that the positive coincidence rate of the assessment reagent and the comparison reagent was 93.60%, the negative coincidence rate was 96.12%, the total coincidence rate was 95.31%, and the Kappa value was 0.8929 > 0.75. Therefore, the detection results of the assessment reagent and the comparison reagent were highly consistent. Through the re-examination of 48 inconsistent samples, 27 cases were positive by the assessment reagent and 21 cases were positive by the comparison reagent. Among the 48 inconsistent samples, 7 were healthy people and patients who were excluded from Aspergillus infection. Among them, 4 were positive by examination reagent and 3 were positive by comparison reagent, which may be the reason of the specificity of reagent itself leading to false positive. 41 cases had symptoms of pulmonary infection, imaging results, microbiological/pathological evidence or antifungal drug use records, etc. 23 cases were positive by examination reagents and 18 cases were positive by comparison reagents, which may be the sensitivity and specificity of reagents themselves leading to false negative.

Stratified statistics were performed on the enrolled samples of this clinical trial, and the statistical results were shown in the following table:

Assessment reagent results for each diagnostic stratification

Diagnostic stratification	Assessment reagent positive	Assessment reagent negative	Total	Assessment reagent sensitivity	Assessment reagent specificity
Proven group	23	5	28	82.14%	/
Probable group	241	37	278	86.69%	/
Possible group	31	204	235	13.19%	/
Control group	40	443	483	/	91.72%
Total	335	689	1024	/	/
The expected population in the control group	29	292	321	/	90.97%

In this clinical trial, 53 cross-samples were detected, including 11 candida-positive serum samples, 11 tuberculobacter IgG antibody positive samples, and 6 mycoplasma pneumonia-igm antibody positive samples. Rheumatoid factor positive serum 4/20 samples in 7 cases, cryptococcus positive samples in 3 cases, systemic lupus erythematosus serum samples in 12 cases, pneumocystis positive serum sample in 1 case, Marneffei cyanobacteria positive serum sample in 1 case, mycoplasma pneumonia-igm antibody and mycobacterium tuberculosis IgG antibody at the same time, the test results of 1 positive serum samples were negative. It can be seen that the assessment reagent has strong anti-interference performance to the cross-pathogen samples.

(3) Experimental conclusions

The test results of the assessment reagent and the comparison reagent were highly consistent, that is, the assessment reagent and the comparison reagent had a high coincidence rate.

(4) GCP compliance and regulatory compliance

Clinical trials in China are conducted in full accordance with the requirements of the "Good Practice for the Quality Management of Medical Device Clinical Trials", that is, China GCP. Clinical trials in China also meet the requirements of the international standard ISO 20916:2019 for clinical studies, and the ISO 20916:2019 compliance checklist is attached.

6.5.2.2 Study on clinical performance of BAL in China

(1) Test method

The purpose of this clinical trial is to evaluate the consistency of diagnostic results between the assessment reagent (this product) and the clinical reference criteria through comparative study, including diagnostic sensitivity, diagnostic specificity, total coincidence rate and Kappa value. The sensitivity, specificity and total coincidence rate of the test results and the diagnostic results of the clinical reference criteria were statistically analyzed by using a 2x2 table, and Kappa consistency analysis was performed. Kappa value ≥0.75 was considered to be highly consistent; 0.4≤Kappa < 0.75 considered consistent; Kappa < 0.4, considered inconsistent.

The samples required for this clinical trial are the remaining alveolar lavage fluid samples screened from the remaining samples of daily testing by the Clinical Laboratory of Jiangxi Provincial People's Hospital. Prospective samples and retrospective samples were included. This clinical trial is intended to be conducted in at least two clinical trial institutions. As there is no established clinical acceptable standard at present, the sample size is calculated in the way that the evaluation indicators meet the expected accuracy level. The calculation formula is as follows:

$$n = \frac{[Z_{1-a/2}]^2 \mathrm{P}(1-\mathrm{P})}{\Delta^2}$$

In the formula, n is the sample size, Z1-a/2 is the quantile of the standard normal distribution of the confidence, P is the expected value of the evaluation index, and △ is the allowable error size of P.

(2) Test results

The clinical trial was conducted in two clinical trial institutions, Jiangxi Provincial People's Hospital and Shandong Provincial Public Health Clinical Center. In this clinical trial, a total of 433 samples were screened, 433 samples were included, 0 cases were dropped, 0 cases were eliminated, and a total of 433 cases were completed. The distribution of 433 samples was as follows: 39 cases in the proven group, 181 cases in the probable group, 53 cases in the possible group, and 160 cases in the control group. Among the 433 samples, 305 were males, accounting for 70.44% of the total sample, and 128 were females, accounting for 29.56%.

Stratified statistics were conducted on the test results of 433 samples, and the statistical results were as follows:

The test results of 199 samples in the proven group and the control group were statistically analyzed. The sensitivity of assessment reagent was 89.74%, and the 95% confidence interval was [76.42%,

95.94%]. The specificity was 96.88%, and the 95% confidence interval was [92.89%, 98.66%]. The total coincidence rate was 95.48%, and the 95% confidence interval was [91.63%, 97.60%]. Kappa value was 0.8579 > 0.75, so the test results were highly consistent with those of the proven group.

The test results of 341 samples in the Probable group and the control group were statistically analyzed. The sensitivity of assessment reagent was 88.95%, and the 95% confidence interval was [83.55%, 92.73%]. The specificity was 96.88%, and the 95% confidence interval was [92.89%, 98.66%]. The total coincidence rate was 92.67%, and the 95% confidence interval was [89.40%, 94.99%]. Kappa value was 0.8536 > 0.75, so the test results were highly consistent with those of the Probable group.

The test results of 380 samples in the proven group, Probable group and control group were statistically analyzed. The sensitivity of assessment reagent was 89.09%, and the 95% confidence interval was [84.28%, 92.56%]. The specificity was 96.88%, and the 95% confidence interval was [92.89%, 98.66%]. The total coincidence rate was 92.37%, and the 95% confidence interval was [89.25%, 94.63%]. Kappa value was 0.8460 > 0.75, so the test results were highly consistent with those of the confirmed and Probable groups.

The detection rate of 53 samples in the possible group was 13.21%. Since the samples in the possible group only had host factors and clinical criteria, but did not have microbial evidence, the detection results were for reference only.

Among the 433 samples, there were 29 cases in which the diagnostic results were inconsistent with the clinical reference standard.

A total of 35 cross-over samples were detected in this clinical trial, including 3 rheumatoid factor positive samples, 2 Acinetobacter baumanniae positive samples, 3 Klebsiella pneumoniae positive samples, 2 Streptococcus pneumoniae positive samples, 1 pneumocystis carinii positive samples, 1 Penicillium Marneffei positive samples, 7 candida positive samples, and 1 Nocardia positive samples. There were 5 positive samples for Pseudomonas aeruginosa, 2 samples from patients with systemic lupus erythematosus, 1 positive sample for Pneumocystis yeli, 6 positive samples for cryptococcus, and 1 positive sample for stenotrophomonas maltophilia. The results showed that a total of 35 cross-samples were tested in this clinical trial, and the test results were negative, no cross-reaction was found.

(3) Experimental conclusions

Through clinical comparison and verification, the sensitivity, specificity, total coincidence rate and Kappa test results of the assessment reagent and the clinical reference standard diagnosis results showed that the consistency between the assessment reagent and the clinical reference standard diagnosis results was good, and the performance of the assessment reagent could meet the clinical requirements for such reagents.

(4) GCP compliance and regulatory compliance

Clinical trials in China are conducted in full accordance with the requirements of the "Good Practice for the Quality Management of Medical Device Clinical Trials", that is, China GCP. Clinical trials in China also meet the requirements of the international standard ISO 20916:2019 for clinical studies, and the ISO 20916:2019 compliance checklist is attached.

6.5.2.3 Peer reviewed literature

6.5.2.3.1 Literature retrieval methods and results

The method of literature retrieval is the same as that of scientific validity literature retrieval, and the specific contents are as follows.

6.5.2.3.1.1 Search date

December 20, 2023

6.5.2.3.1.2 Literature retrieval personnel profile

Yuan Zhang obtained a Ph.D. in Chemical Biology from Nankai University in 2019.In 2021, she completed postdoctoral research work in the Light Industry Technology and Engineering program at Tianjin University of Science and Technology. Since August 2018, she has been working at Dynamiker Biotechnology (Tianjin) Co., Ltd. and she is a Senior Manager in the Clinical Medicine Department and Head of the Overseas Registration Department.

She participated in 10 multicenter studies and 32 single center studies conducted by Dynamiker in key hospitals in China. She has participated in the clinical registration of multiple products in China and has successfully obtained 13 NMPA Class III registration certificates. She participated in writing and publishing over 20 academic articles, and participated in the writing of expert consensus, guidelines, and industry standards in China. During the COVID-19 epidemic, she won one special project of China Postdoctoral Science Foundation for the prevention and control of COVID-19, and has rich experience in clinical transformation and product registration in the field of in vitro diagnosis of fungal serology.

Yanting Wang obtained a master's degree in Microbiology and Biochemical Pharmacy from Tianjin University of Science and Technology. She has a Chinese Western Medicine Practicing Pharmacist Qualification Certificate. Since January 2023, she has been working at Dynamiker Biotechnology (Tianjin) Co., Ltd.as an overseas registered engineer.

She has worked at the Biological Testing Laboratory of the National Institute of Nanotechnology and Engineering, with one year of experience in the research and development of in vitro diagnostic reagents. During her tenure at Tianjin International Joint Academy of Biomedicine, she mainly conducted research on pharmaceutical policies and regulations, and wrote and published over 20 Chinese and English papers and research reports. She visited and studied at Department of Regulatory and Quality Sciences in Alfred E. Mann School of Pharmacy and Pharmaceutical Sciences at University of Southern California from July to August 2019.

6.5.2.3.1.3 Data source

Data source: MEDLINE Pubmed, Embase

Search scope: system search

6.5.2.3.1.4 Search time range

Literature related to the past 10 years was searched through MEDLINE Pubmed and Embase databases.

6.5.2.3.1.5 Retrieve the media used

All data is searched online in the following ways:

Data source	Acquisition mode
MEDLINE Pubmed	https://pubmed.ncbi.nlm.nih.gov/
Embase	http://www.embase.com

6.5.2.3.1.6 MEDLINE Pubmed data retrieval strategy

According to the document retrieval strategy recommended in Annex A5 of MEDDEV 2.7/1 revision 4 of the EU IVDR Regulation, the PICO principles (patient characteristics, type of intervention, control and outcome) were used for retrieval analysis.

(1). P(patient characteristics) search term selection and search style

The intended population is defined in the product description as follows: The product is intended to assist in the early diagnosis of Invasive Aspergillosis (IA). Therefore, invasive Aspergillosis was selected as the search keyword, and the keyword was queried as subject word and free word. The search process was as follows:

#1："Invasive Pulmonary Aspergillosis"[Mesh]

#2：((invasive pulmonary aspergillosis[Title/Abstract]) OR (pulmonary aspergill*[Title/Abstract])) OR (invasive aspergill*[Title/Abstract])

P(patient characteristics) search formula is as follows:

#3: #1 OR #2

(2). I (type of intervention) search term selection and search style

The analyte of this product is aspergillus galactomannan. Therefore, subject and free word queries were conducted in MEDLINE Pubmed with the keyword Galactomannan. The search process is as follows:

#4："galactomannan" [Supplementary Concept]

#5：Galactomannan [Title/Abstract]

I (type of intervention): galactomannan search formula is as follows：

#6: #4 OR #5

The methodology of this product is sandwich enzyme-linked immunosorbent. Aspergillus Platelia produced by Bio-Rad and IMMY can be used as the equivalent device of this product. Therefore, the four terms Dynamiker, Bio-Rad, IMMY and Platelia were queried in MEDLINE Pubmed.

I (type of intervention) : methodological search is as follows：

#7：(((dynamiker) OR (Bio-rad)) OR (platelia)) OR (IMMY)

(3). Total search mode

#8: #3 AND #6 AND #7，The search period is limited to 2003/5/16 to 2023/12/20.

6.5.2.3.1.7 Embase database retrieval strategy

PICO principles (patient characteristics, intervention type, control and outcome) were used for retrieval analysis.

(1).P(patient characteristics) search term selection and search style

Invasive Aspergillosis was selected as the search keyword. The invasive Aspergillosis was searched in embase according to the free words identified on the keyword in pubmed. The search process was as follows: Therefore, the search style of P(patient characteristics) was as follows:

#1：'invasive aspergillosis'/exp

#2：'pulmonary aspergill*':ti,ab,kw OR 'invasive aspergill*':ti,ab,kw

P(patient characteristics) search mode is as follows:

#3：#1 OR #2

(2).I (type of intervention) search term selection and search style

This product is used for the detection of galactomannan, so Galactomannan was used as the key word to search in embase. The search process is as follows:

#4：'galactomannan'/exp

#5：galactomannan:ti,ab,kw

I (type of intervention)中galactomannan的检索式如下：

#6：#4 OR #5

The methodology of this product is sandwich enzyme-linked immunosorbent. Aspergillus Platelia produced by Bio-Rad and IMMY can be used as the equivalent device of this product. Therefore, the four terms of Dynamiker, Bio-Rad, IMMY and Platelia are queried in Embase. I (type of intervention) methodology search mode is as follows:

#7：dynamiker OR 'bio-rad' OR platelia OR immy

(3). Total search mode

The search period is limited from May 16, 2003/to December 20, 2023/20. Since the publication year filter of Embase cannot be specific to the month date, the time range of search is selected from 2003 to 2023, and the literature that is not within the limited search scope is excluded during the initial screening. The overall search mode is as follows:

#8: #3 AND #6 AND #7 AND [2003-2023]/py

6.5.2.3.1.8 **Database search result**

A total of 124 articles were obtained through Pubmed database, and 280 articles were obtained through Embase database.

Data source	Search mode	Search period	Search date	Search results
MEDLINE Pubmed	#3 AND #6 AND #7	2003.05.16-2023.12.20	2023.12.20	124
Embase	#3 AND #6 AND #7 AND [2003-2023]/py	2003-2023	2023.12.20	280

6.5.2.3.2 Literature screening, inclusion and exclusion criteria

The literatures obtained through PubMed and Embase database were imported into Endnote literature management software, and duplicate literatures were removed. After de-duplication, the first round of screening was carried out according to the title and abstract of the literature, and the second

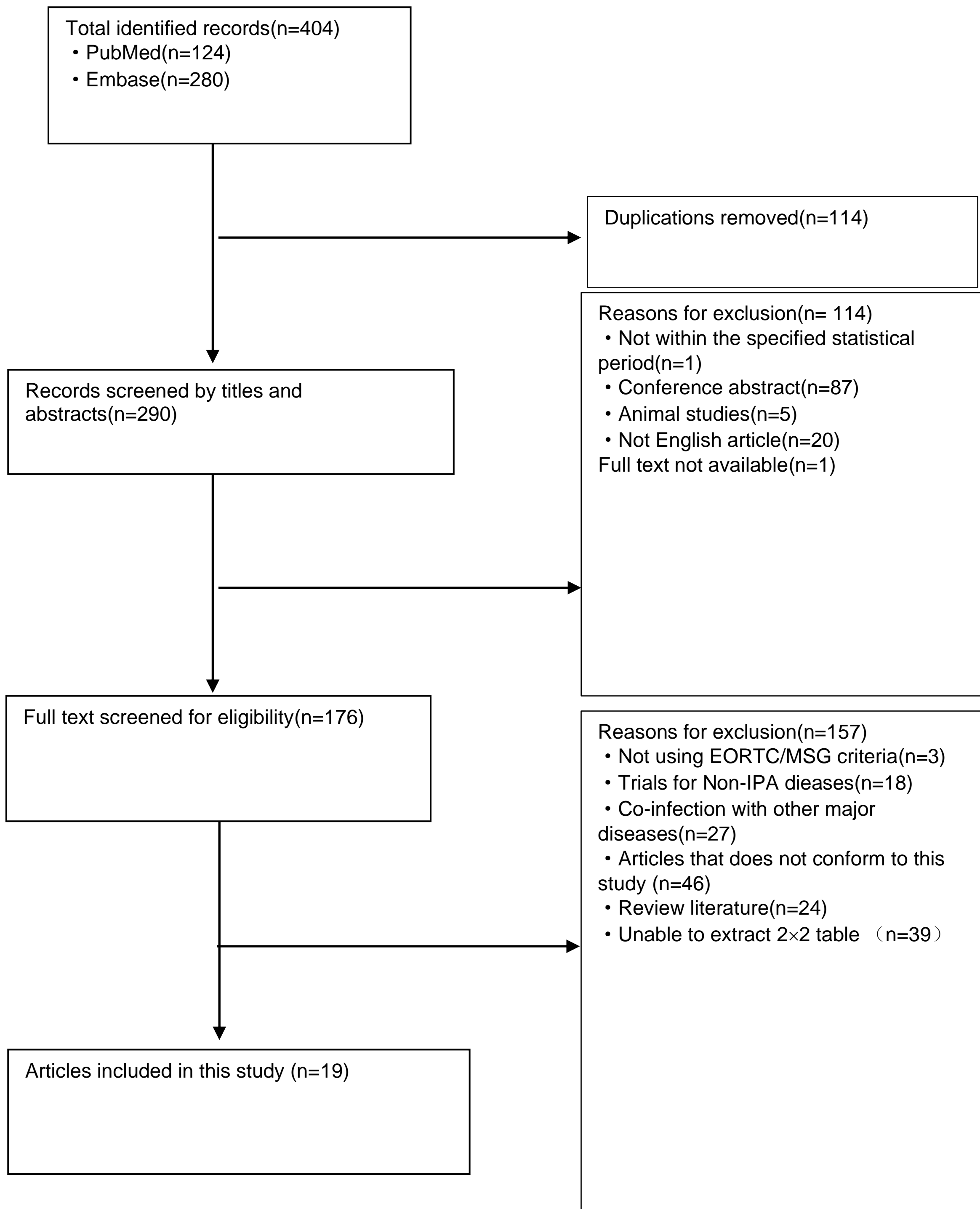
round of screening was carried out according to the full text of the literature. The inclusion criteria were: (1) published literature with full text available; (2) research subjects were patients suspected of invasive pulmonary aspergillosis who were tested with serum or alveolar lavage fluid using this product or its equivalent; and (3) IPA diagnostic criteria were IPA diagnostic criteria formulated by EORTC/MSG or similar criteria. (4) The gold standard for diagnosis was positive group was confirmed and clinically diagnosed IPA, negative group was non-IPA, and the suspected IPA group was excluded. (5) The data were complete and four-cell tables could be extracted

In the first round of screening, the following types of literature were excluded: (1) literature that was not within the set time range for scientifically valid literature search, (2) conference abstracts, (3) non-clinical research literature whose research type was animal testing, (4) non-English literature, and (5) literature whose full text was not available.

The second round of screening was conducted based on the full text content, and the following types of documents were excluded: (1) Review literature, (2) the patient population discussed in the literature is not invasive pulmonary aspergillosis, (3) The patient population discussed in the literature has COVID-19, influenza virus infection, major bacterial or viral co-infection, ICU severe disease and other major diseases. (4) The diagnostic criteria for IPA did not adopt the diagnostic criteria for IPA formulated by EORTC/MSG or similar criteria; (5) the studies that included the proposed IPA into the gold standard positive group or gold standard negative group; (6) the studies could not effectively extract the data of diagnostic sensitivity and specificity; (6) the data were incomplete and the four-cell table could not be extracted; (7) Literature whose research objects and topics are inconsistent with the analysis of this study.

6.5.2.3.3 Literature screening results

According to the criteria for inclusion and exclusion of literatures, the screening process is shown in Figure 1. Finally, a total of 19 literatures were included in clinical research analysis. See Annex 1 for the list of literatures after re-weight removal and the reasons for exclusion in the first and second rounds of screening. See Annex 2 for the list of literatures finally included in the scientific validity analysis.



6.5.2.3.4 Evaluation of Methodological quality of clinical research literature

Referring to 9.3.1 in MEDDEV 2.7/1 and 6.3 in MDCG 2020-6, the included clinical literature was a diagnostic accuracy study, and the Quadas-2 quality evaluation method was adopted.

(1) Introduction of Quadas-2 evaluation methods

QUADAS is a tool specifically designed to evaluate the quality of original studies in systematic review/meta-analysis of diagnostic accuracy tests developed by Penny Whiting et al., University of York, UK, in accordance with Delphi method in 2003 [1,2]. Therefore, it has been widely used and

recommended since its launch in 2003. Agency for Health Research and Quality, Cochrane Collaboration, U.K.National Institute for Health and Clinical Excellence and other institutions recommend using QUADAS tools to evaluate research quality when making systematic reviews/meta-analyses of diagnostic accuracy tests [3]. As the practice continued to deepen, the QUADAS research and development team revised it, launching a revised version of QUADAS-2 in 2011 [4].

The QUADAS-2 tool consists of four main components: case selection, trials to be evaluated, gold criteria, and case flow and progression (see Table 1). All components are assessed for risk of bias, and the first three components are also assessed for clinical applicability. These research design questions are related to the potential of bias and are intended to help evaluators judge the risk of bias. However, the judgment of clinical applicability did not take into account the landmark issues.

The "yes", "no" or "uncertain" answers to the relevant signature questions included in each section may correspond to a bias risk rating of "low", "high" or "uncertain". If the answer to all the signature questions in a range is "yes", then the risk of bias can be assessed as low; If the answer to one of all information questions is "no", then there is a possibility of bias. The "uncertain" rating refers to the fact that the literature does not provide detailed information that makes it difficult for the reviewer to make a judgment, and it can only be used if the reported data is insufficient.

Table 1. Risk of Bias and Applicability Judgments in QUADAS-2

Domain	Patient Selection	Index Test	Reference Standard	Flow and Timing
Description	Describe methods of patient selection Describe included patients (previous testing, presentation, intended use of index test, and setting)	Describe the index test and how it was conducted and interpreted	Describe the reference standard and how it was conducted and interpreted	Describe any patients who did not receive the index tests or reference standard or who were excluded from the 2 ×2 table (refer to flow diagram) Describe the interval and any interventions between index tests and the reference standard
Signaling questions (yes, no, or unclear)	Was a consecutive or random sample of patients enrolled? Was a case–control design avoided? Did the study avoid inappropriate exclusions?	Were the index test results interpreted without knowledge of the results of the reference standard? If a threshold was used, was it prespecified?	Is the reference standard likely to correctly classify the target condition? Were the reference standard results interpreted without knowledge of the results of the index test?	Was there an appropriate interval between index tests and reference standard? Did all patients receive a reference standard? Did all patients receive the same

				reference standard? Were all patients included in the analysis?
Risk of bias (high, low, or unclear)	Could the selection of patients have introduced bias?	Could the conduct or interpretation of the index test have introduced bias?	Could the reference standard, its conduct, or its interpretation have introduced bias?	Could the patient flow have introduced bias?
Concerns about applicability (high, low, or unclear)	Are there concerns that the included patients do not match the review question?	Are there concerns that the index test, its conduct, or its interpretation differ from the review question?	Are there concerns that the target condition as defined by the reference standard does not match the review question?	

(2) Characteristics of clinical literatures included

①Serum

The clinical research characteristics of the included clinical literature for serum GM detection were analyzed, as shown in Table 2.

In Table 2, all study characteristics of the included studies about serum galactomannan are presented. In total, 12 studies were included with a sample size of 2305 patients including 287 patients with proven or probable IPA and 126 patients with possible IPA. All study populations comprised adult patients with a hematological malignancy, patients with hematopoietic stem cell transplantation, adults with hematologic disorders receiving piperacillin/tazobactam, and immunocompromised patients. The EORTC/MSG criteria of 2008 and 2002 were most commonly used and there was only one study that used the newest update from 2020. The most common ODI cut-off value was 0.5.

Table 2. Serum Galactomannan Study Characteristics of studies included in Methodological quality

No	Study	Country	Data collections	Study design	Sampling method	Patient population	Median age: year (range)	Sample size (N=)**	proven/ probable	Number of possible	Diagnostic criteria	Cut-off	Type of IA
20	Lai C C 2007	China	NA	Case-control	consecutive	with risk for IPA	54(12-76)	189	14	26	EORTC/MSG 2002	≥1.5	with risk for IPA
47	Held J 2013	Germany	Prospective	Case-control	consecutive	with risk for IPA	54(21-70)	101	10	20	EORTC/MSG 2008	≥0.5	invasive aspergillosis following haematopoietic stem cell transplantation
76	Pinel C 2003	France	Prospective	Case-control	consecutive	with risk for IPA	NA	807	34	22	EORTC/MSG 2002	≥1.0	with risk for IPA
94	Boch T 2015	Germany	Restrospective	Cohort	consecutive	with risk for IPA	57 (22-79)	34	26	0	EORTC/MSG 2008	≥0.5	Immunocompromised patients
119	Roiz-Mesones M P 2023	Spain	Restrospective	Case-control	unclear	with risk for IPA	NA	64	27	0	EORTC/MSG 2020	≥0.5	with risk for IPA
125	Alhambra A 2007	Spain	Prospective	Cohort	unclear	with risk for IPA	52 (16-79)	78	9	0	EORTC/MSG 2002	≥0.5	adult hematological patients treated with piperacillin-tazobactam
174	Gao X 2010	China	Prospective	Case-control	consecutive	Suspected of IPA	Mean age: 65.3	261	5	2	EORTC/MSG 2008	≥1.5	patients diagnosed with AECOPD
199	Maertens J A 2007	France	Restrospective	case-control	consecutive	with risk for IPA	Range:16–76	239	38	0	EORTC/MSG 2002	≥1.5; ≥1.0; ≥0.5	adult hematology patients
244	KUMAR J 2018	India	NA	case-control	unclear	Suspected of IPA	NA	74	14	3	EORTC/MSG 2008	1.5	Pediatric Febrile Neutropenia

				study									
245	Wang L 2014	China	Restrospective	case-control study	consecutive	with risk for IPA	(64.94± 13.34)	80	34	0	EORTC/MSG 2008	0.5	with risk for IPA
271	Sulahian A 2014	France	NA	case-control study	consecutive	with risk for IPA	/	216	69	0	EORTC/MSG 2008	0.5	patients with hematological malignancies
278	TĂNASE A D 2012	Romania	Restrospective	case-control study	consecutive	with risk for IPA	/	162	7	53	EORTC/MSG 2002	0.5	hematopoietic stem cell transplantation

②BAL

In Table 3, all study characteristics of the included studies about BAL galactomannan are presented. In total, 7 studies were included with a sample size of 636 patients including 216 patients with proven or probable IPA and 73 patients with possible IPA. All study populations comprised adult (18+) patients with a hematological malignancy, hematopoietic stem cell transplant patients, lung transplant recipients and Immunocompromised patients. The EORTC/MSG criteria of 2020 were most commonly used. The most common ODI cut-off value were 0.5 and 1.0.

Table 3. BAL Galactomannan Study Characteristics of studies included in Methodological quality

No	Study	Country	Data collections	Study design	Sampling method	Patient population	Mean age: year (range)	Sample size (N=)**	proven/ probable	Number of possible	Diagnostic criteria	Cut-off used	Type of IA
13	Musher B 2004	USA	Prospective	Case-control	unclear	with risk for IPA	Mean age：45.2	99	49	0	EORTC/MSG 2002	≥0.5；≥1.0；	hematopoietic stem cell transplant patients
49	Luong M L 2011	USA	Restrospective	Case-control	unclear	with risk for IPA	Median age：54.7	150	16	0	EORTC/MSG 2008	≥0.5；≥1.0；	Lung Transplant Recipients
94	Boch T 2016	Germany	Restrospective	Cohort	consecutive	with risk for IPA	57 (22-79)	34	26	0	EORTC/MSG 2008	≥0.5	Immunocompromised patients
119	Roiz-Mesones M P 2023	Spain	Restrospective	Case-control	unclear	with risk for IPA	NA	28	9	0	EORTC/MSG 2020	≥1.0	with risk for IPA
204	Egger M 2022	USA	Restrospective	Cohort	unclear	Suspected of IPA	Median age : 62 (46–86)	115	43	10	EORTC/ MSG 2020	≥1.0	Suspected of IPA
205	McKinney W P 2022	New Zealand	unclear	Case-control	unclear	with risk for IPA	NA	69	7	0	EORTC/ MSG 2020	≥1.0; ≥1.5; ≥0.5	

246	Buil J B 2023	Netherlands	Restrospective	case-control study	unclear	Suspected of IPA	Median age：64 (55–70)	141	66	63	EORTC/MSG 2020	≥1.0; ≥0.5	Patients with Hematological Disease

(3) Quadas-2 evaluation results of the included literature

①The results of the included clinical literature were evaluated for serum GM detection

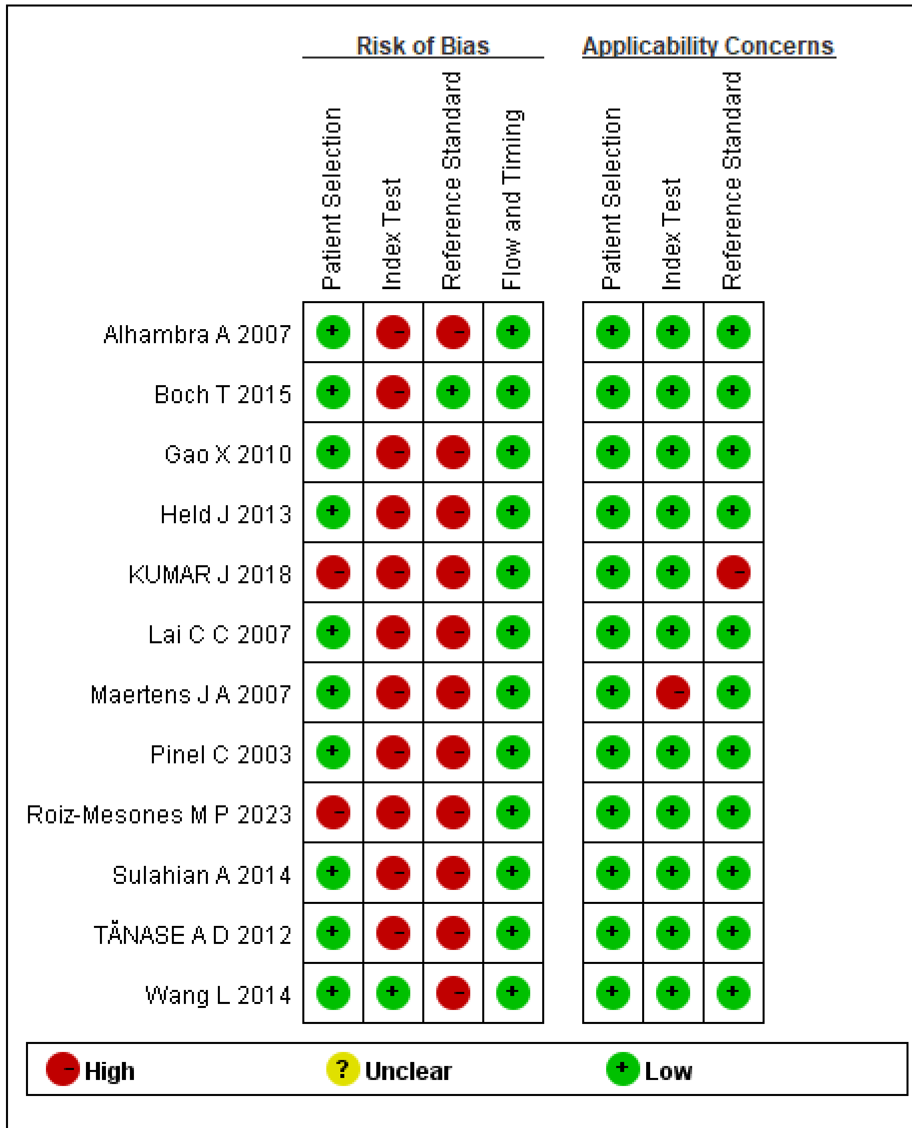


Figure 2. **Detailed methodological quality assessment of serum studies using the QUADAS-2 tool**

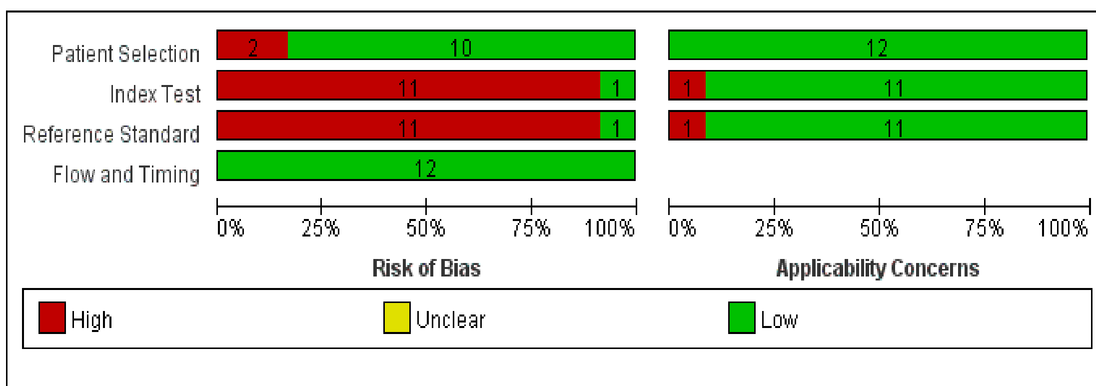


Figure 3. **Overall methodological quality assessment of serum studies using the QUADAS-2 tool**

②The results of the included clinical literature were reviewed for GM testing of alveolar lavage fluid

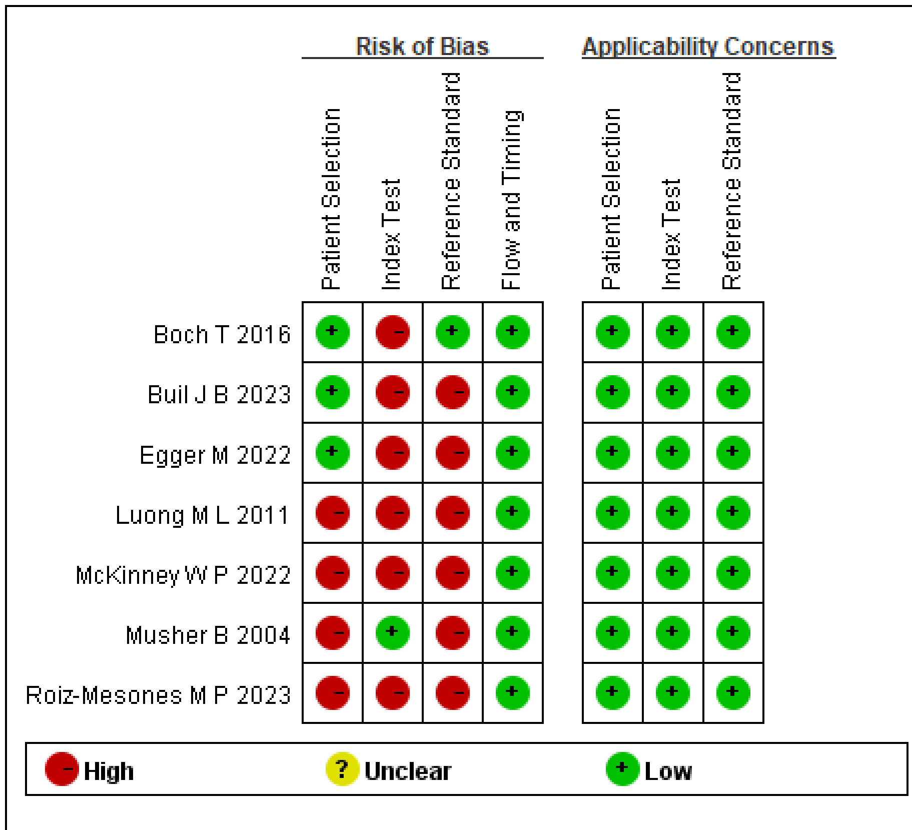


Figure 4. Detailed methodological quality assessment of BAL studies using the QUADAS-2 tool

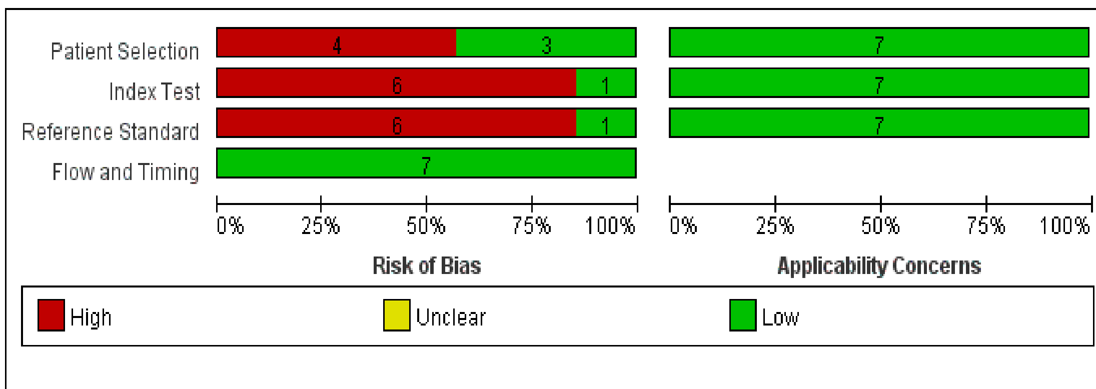


Figure 5. **Overall methodological quality assessment of BAL studies using the QUADAS-2 tool**

6.5.2.3.5 Clinical research literature correlation (revelance) evaluation

According to the requirements of clinical data correlation evaluation in 9.3.2 of MEDDEV 2.7/1 and combined with the evaluation results of Quadas, the clinical literature was classified into pivotal data sets and other data sets. Because the included literature is all pivotal performance data based on equivalent devices and has the same intended use as the product, all of the literature is included in the pivotal data dataset.

6.5.2.3.6 Evaluation of clinical research literature contribution

According to the contribution evaluation requirements of clinical data set in MEDDEV 2.7/1, the pivotal data set was classified into systematic review, meta-analysis study, cohort study and case-control study data set. According to the clinical evidence level of evidence-based medicine, Systematic

review/ META analysis has the highest evidence level, which is level I, Cohort study is level II, and Case control study is level III.

Among them, the systematic review and meta-analysis data set included two literatures, which were numbered as L86 and L143;

The cohort study dataset included 3 articles, which were numbered 94, 125 and 204.

The data set of the case-control study included 14 articles, which were numbered 13, 20, 47, 49, 76, 119, 174, 199, 205, 244, 245, 246, 271, 278.

6.5.2.3.7 Safety and performance evaluation of clinical research literature

Every article in every dataset in the pivotal data dataset is evaluated based on security or performance.

6.5.2.3.8 Clinical performance analysis of systematic review and meta-analysis datasets

Two systematic reviews and meta-analysis studies on serum galactomannan have been included, see Table 5 (Husain 2007, Leeflang 2015). All included studies of Husain 2007 and Leeflang 2015 used Bio-Rad Platelia sandwich ELISA test.

Husain 2007 focused mainly on haematological patients an included 27 studies. At a cut-off level of 0.5, the sensitivity was found to be 0.79 and the specificity 0.85. At a cut-off level of 1.0, the sensitivity was found to be 0.65 and the specificity 0.94. At a cut-off of 1.5 sensitivity dropped to 0.48 and specificity rose to 0.95.

Leeflang 2015 focused specifically on immunocompromised patients and included 50 studies. At a cut-off level of 0.5, the sensitivity was found to be 0.82 and the specificity 0.81. At a cut-off level of 1.0, the sensitivity was found to be 0.72 and the specificity 0.88. At a cut-off of 1.5 sensitivity dropped to 0.61 and specificity rose to 0.97.

Table 4. Characteristics and results of Meta-analysis studies

Meta-analysis review	Number of studies	Patient population	Cut-off ODI	Sensitivity (95% CI)	Specificity (95% CI)
Husain 2007	27	Patients with hematological malignancy, bone marrow transplant recipients, immunocompromised, solid-organ transplant recipients, Bone marrow transplant recipients	0.5	0.79 (0.69–0.87)	0.86 (0.83–0.89)
			1	0.65 (0.57–0.72)	0.94 (0.92–0.95)
			1.5	0.48 (0.41–0.56)	0.95 (0.93–0.96)
Leeflang 2015	50	immunocompromised patients	0.5	0.82 (0.73-0.90)	0.81 (0.72-0.90)
			1	0.72 (0.65-0.80)	0.88 (0.84-0.92)

	1.5	0.61 (0.47-0.75)	0.93 (0.89-0.97)

CI: confidence interval; ODI: optical density index

6.5.2.3.9 Clinical performance analysis of cohort study dataset

Table 5. Serum Galactomannan Study Results

No (year)	Proven/probable vs no IA						Cut-off
	TP	FP	TN	FN	Sensitivity [95%CI]	Specificity [95% CI]	
94	6	1	7	20	23	88	0.5
125	9	8	61	0	100.0	88.4	0.5

Table 6. BAL Galactomannan Study Results

No (year)	Proven/probable vs no IA						Cut-off
	TP	FP	TN	FN	Sensitivity [95%CI]	Specificity [95% CI]	
94	22	1	7	4	85	88	0.5
204	52	2	45	6	90	96	0.5

6.5.2.3. Clinical performance analysis of case-control study dataset

Table 7. Serum Galactomannan Study Results

No (year)	Proven/probable vs no IA						Cut-off
	TP	FP	TN	FN	Sensitivity [95%CI]	Specificity [95% CI]	
20	11	9	3	140	78.6	93.9	1.5
47	4	10	81	6	40.0 (13.7-72.6)	89.0 (80.3-94.3)	0.5
76	17	3	748	17	50.0	99.6	1.0
119	24	3	34	3	88.9	91.9	0.5
119	16	3	34	11	43.2	91.9	0.5
199	29	5	196	9	76.3 (59.8-88.6)	97.5 (94.3-99.2)	1.5
199	31	7	194	7	81.6 (65.7-92.3)	96.5 (93.0-98.6)	1.0
199	37	19	182	1	97.4 (86.2-99.9)	90.5 (85.6-94.2)	0.5
244	11	33	30	0	100	47.7	0.5
244	9	21	42	2	81.8	66.7	1.0
244	9	5	58	2	81.8	92.1	1.5
245	18	9	37	16	52.94 (35.4-69.8)	80.43 (65.6-90.1)	0.5
271	34	5	142	35	49.3	96.6	0.5

Table 8 BAL Galactomannan Study Results

No (year)	Proven/probable vs no IA						Cut-off
	TP	FP	TN	FN	Sensitivity [95%CI]	Specificity [95%	

						CI]	
13	37	3	47	12	76 (61–87)	94 (84–99)	0.5
13	30	1	49	19	61 (46–75)	98(89-100)	1.0
49	14	15	118	1	93 (68–100)	89 (82–93)	0.5
49	10	4	129	5	67 (38–88)	97 (92–99)	1.0
119	9	6	13	0	100.0	68.4	1.0
119	8	3	16	1	88.9	84.2	1.0
174	4	15	239	1	80.0	94.1	1.5
205	5	11	51	2	71	82	0.5
205	5	7	55	2	71	89	1.0
205	5	5	57	2	71	92	1.5
246	51	1	11	15	74 (64–84)	92 (65–100)	0.5
246	42	0	12	24	63 (52–74)	100 (76-100)	1.0
278	6	7	148	1	85.7	91.3	0.5

6.5.2.3.11 Intended use and population analysis

(1) Serum

A total of 12 clinical studies were included in the peer-reviewed literature based on serum testing. Three of them were from China, including 530 cases. 8 articles were from France, Germany, Spain and other European countries, including 1701 cases; One study from India included 74 cases.

In these literatures, the intended use of GM testing is for testing with IPA risk, and the prevalence of the tested population in each literature is shown in the table below.

No	Study	Type of patient
20	Lai C C 2007	blood diseases mainly
47	Held J 2013	alloHSCT
76	Pinel C 2003	AML(acute myeloid leukemia), NHL(non-Hodgkin’s lymphoma), CLL (chronic lymphocytic leukemia) mainly
94	Boch T 2015	AML, Malignant lymphoma, Neutropenia mainly
119	Roiz-Mesones M P 2023	MHD(malignant hematological disease) mainly
125	Alhambra A 2007	NHL(Non-Hodgking’s lymphoma), MM(multiple Myeloma), AML mainly
174	Gao X 2010	acute exacerbation of COPD
199	Maertens J A 2007	alloHSCT, ALL(acute lymphoblastic leukemia) mainly
244	KUMAR J 2018	febrile neutropenia
245	Wang L 2014	The patients were mainly from intensive care and geriatrics, the diagnosis was not mentioned.
271	Sulahian A 2014	AML/MDS, CLD(chronic lymphoproliferative disorder), ALL mainly
278	TĂNASE A D 2012	HSCT

(2)BAL

A total of 7 clinical studies were included in the peer-reviewed literature based on serum testing. Among them, 3 articles were from North America, including 364 cases. Three articles were from Europe, including 203 cases. One review was from Europe, including 69 cases.

In these literatures, the intended use of GM testing is for testing with IPA risk. The prevalence of the population tested in each literature is shown in the table below.

No	Study	Type of patient
13	Musher B 2004	HSCT
49	Luong M L 2011	The patients were lung transplant recipients，the diagnosis was mainly Cystic fibrosis and COPD/emphysema.
94	Boch T 2016	AML, Malignant lymphoma, Neutropenia mainly
119	Roiz-Mesones M P 2023	MHD (malignant hematological disease) mainly
204	Egger M 2022	Hematologic malignancy, ICU patient mainly
205	McKinney W P 2022	Not mentioned.
246	Buil J B 2023	AML, alloHSCT mainly

6.5.2.5 Published public experience

6.5.2.5.1 Foreign laboratory comparison study (this product VS equivalent equipment)

(1)Rome, Italy: Evaluation of Dynamiker Aspergillus Galactomannan assay

a) Protocol

From 12th April 2019, we started evaluation of Dynamiker Aspergillus Galactomannan assay comparing with Bio-Rad Platelia Aspergillus Galactomannan Ag kit, using routine clinical samples as well as known samples from Reference lab (CAMPIONI PROVENIENTI DAL GEMELLI).

In total, 31 samples including 8 serum and 22 BAL samples are tested by both Dynamiker and Bio-Rad. Sample NO. 29, NO. 30 and NO. 31 are known positive samples from Gemelli hospital.

b) Result

Among the 31 samples, Dynamiker gave 5 positives and 26 negative result, and Bio Rad gave 4 positive and 27 negative results. Dynamiker result are concordance with Bio-Rad in 30 samples. The only difference is Sample 5, which Dynamiker give positive (0.55) and Bio-Rad gave negative (0.14). The Aspergillus PCR result of this sample was positive. The positive concordance rate, negative concordance rate and total concordance rate of Dynamiker and Bio-Rad is 100%, 96.29% and 96.77%, respectively.

Table 1 Statistical data

Statistical data		Bio-Rad Platelia Galactomannan		
		Positive	Negative	Subtotal
Dynamiker Galactomannan	Positive	4	1	5
	Negative	0	26	26
	Subtotal	4	27	31

c) Conclusion

Dynamiker Aspergillus Galactomannan assay shows good agreement with Bio-Rad Platelia Aspergillus Galactomannan test kit. Further study should be done to get more performance data.

(2) KINGMED: Clinical study of Dynamiker Aspergillus Galactomannan assay (Sandwich)

a) Protocol of the study

1. Double-blind test 2. In total there are 218 clinical samples enrolled in this study, of which there are 181 serum and 37 BAL samples. 3. All these samples were tested by both Dynamiker GM assay (Sandwich) and Bio-Rad Platelia GM assay at the same time. 4. The conformity of both assays were calculated.

b) Data analysis

Considering Dynamiker GM assay and Bio-Rad Platelia GM assay as Test assay and Control assay, respectively, calculate the positive conformity rate, negative conformity rate and total conformity rate.

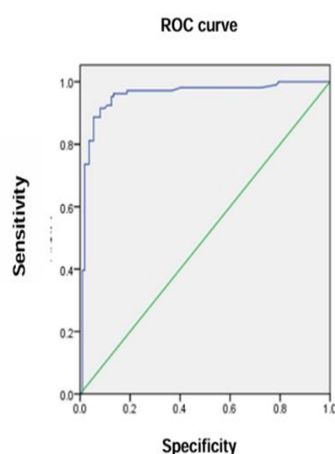
c) Result

1. Conformity results.

		Bio-Rad GM		
		Positive	Negative	Total
Dynamiker GM	Positive	94	9	103
	Negative	4	111	115
	Total	98	120	218

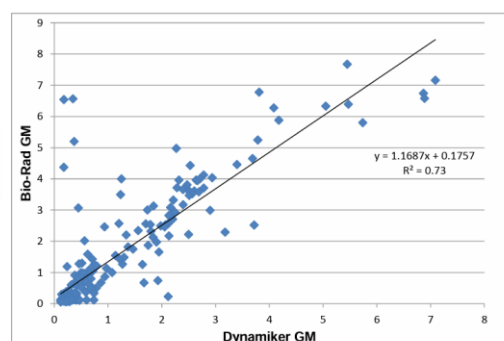
- **Positive conformity rate = 94/103×100% = 91.26%**
- **Negative conformity rate = 111/115×100% = 96.52%**
- **Total conformity rate = (94+111)/218×100% = 94.04%**

2. ROC analysis



AUC: 0.965

3. Correlation analysis
Plot scatter diagram with Test assay result as X-axis, and Control assay result as Y-axis, calculate correlation coefficient r.



The AUC is 0.965 showing the good conformity of the two assays. Linear regression equation is Y=1.1687x + 0.1757, R^2=0.73.

d) Conclusion

This showing Dynamiker Aspergillus Galactomannan assay (Sandwich) has good conformity with Bio-Rad Platelia Aspergillus Galactomannan assay.

6.5.2.5.2 Foreign laboratory comparison study (This product VS clinical standard)

(1) Brazil: VALIDATION PROTOCOL Dynamiker Aspergillus Galactomannan Assay

a) Purpose

assessment and performance of the DNK Aspergillus Galactomannan kit and the Platelia Aspergillus EIA in serum from adult neutropenic oncohaematological patients with a highly expect prevalence of invasive aspergillosis.

Clinical data and sera will be collected prospectively and patients classified according to the European Organization for Research and Treatment of Cancer (EORTC)/Mycoses Study Group (MSG) 2008 guidelines.

b) Results

Clinical testing to evaluate the sensitivity, specificity and predictive value of the DNK Aspergillus Galactomannan was conducted on adult neutropenic oncohaematological patients using a total of 58 patients from the following populations:

Patients without signs of Invasive Aspergillosis (control patients)

Patients with probable Invasive Aspergillosis

Patients with proven Invasive Aspergillosis

①Sensitivity: - Proven Aspergillosis: was 81.8% (95% Confidence Interval 52.3 – 94.9%); - Probable Aspergillosis: was 77.8% (95% Confidence Interval 54.8 – 91.0%); - Combined Proven and Probable Aspergillosis: 79.3% (95% Confidence Interval 61.6 – 90.2%)

②Specificity: the test showed 80.5%

③Reproducibility: the results from the standards and controls were generally reproducible between days. Some variability in results was seen with the standard “a” and “b”.

c) Conclusions

- The performance characteristics of Platelia Aspergillus EIA and DNK Aspergillus Galactomannan kit were similar for these tests. - Prospective studies are needed to determine more value of the new assay.

(2) Corelation between Antifungal Treatment and Galactomannan Antigen in Adult Hematologic Patients at Risk for Invasive Aspergilosis

a) Objectives

To analyse the correlation between antifungal treatment and galactomannan antigen in adult hematologic paients at risk for invasive aspergilosis (IA) together with the results of serial serum Aspergillus galactomannan (GM) antigen testing.

b) Material and methods

In a retrospective study for patients at high risk of aspergilus pulmonary infection, serum GM test was used to detect GM concentration 2-3 times per week during the periods of high risk for IA. High-resolution CT was performed in case of abnormal chest X-ray and/or persistent fever after 5 days of antibiotic treatment. IA was classified as either "proven" or "probable” in accordance with the definitions stated by the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC-MSG).

c) Results

A total of 82 hematological patients were diagnosed of "proven IA" (n=1), and "probable” IA (n=28), and "possible IFD" (n=23'and "No IFD" (n=30). The sensitivity of the GM test was 84.6%, and the specificity was 81.3%. The false positive rate was 18.8%, the false negative rate 15.4% and the diagnosis rate 82.8%. This group of 82 patients received prophylaxis fluconazole treatment at a median of days 19.7 (range 9- 26).

d) Conclusion:

Serum GM test could be taken 2-3 times/week in adult hematologic patients at risk for IA. The GM value was correlated to the amount and the fungal load in patients. The GM test is also earlier than the conventional CT or chest X-ray scan.

Group	Diagnosis before GM test	GM (+)	GM (-)
Proven IA	1	1	0
Probable IA	28	22	6
Possible IFI	23	5	18
No IFI	30	4	26
Total	82	32	50

Table 2. GM test results and patient diagnosis

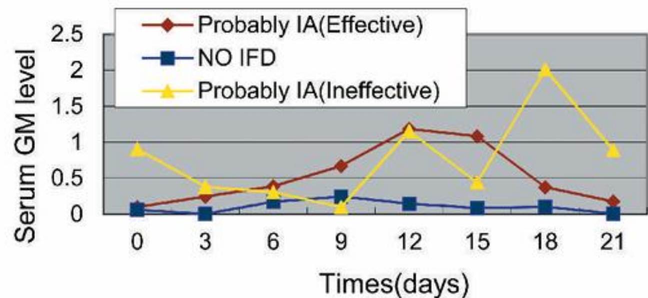


Table 3. GM test results during the antifungal treatment

6.6. Ongoing or planned post-market performance follow-up

Post-Market Surveillance for the product Dynamiker Aspergillus Galactomannan Assay does not indicate any changes necessary for the Performance Evaluation according to (EU) 2017/746 IVDR Article 56 and Part A of Annex XIII.

No need for any preventive and/or corrective measures has been identified for the product in scope of this report. The safety and performance of the device throughout its expected lifetime can be confirmed and the continued acceptability of the clinical evidence and of the benefit-risk ratio is ensured.

7. Metrological traceability of assigned values

7.1. Explanation of the unit of measurement, if applicable.

This product has no primary standard and primary reference measurement procedures, nor international agreed reference measurement procedures and international agreed standards, and cannot be traceable to SI in measurement, so it is not applicable.

7.2. Identification of applied reference materials and/or reference measurement procedures of higher order used by the manufacturer for the calibration of the device.

This product is a qualitative assay. The interpretation of the test results is based on the I-value (the ratio of the sample OD value to the mean OD value of the cut-off QC), so the test performance depends on the cut-off QC to a certain extent. However, since there is no international or national standard product for the metrological traceability of the cut-off QC, the self-made Aspergillus galactomannan antigen was used to establish the internal measurement procedure of the enterprise. The

galactomannan was obtained by culturing ATCC standard Aspergillus strains, and its purity was determined to be more than 90% by HPLC. No impurity protein bands were analyzed by SDS-PAGE, and the concentration of galactomannan was determined by the Dubois-phenol sulfate method to achieve traceability.

8. Suggested profile and training for users

The product is intended for in vitro diagnostic use by trained professionals only. In case of manual application, professional users are any personnel who are qualified to perform IVD examinations through special education and training.

9. Revision history

SSP revision number	Date issued	Change description	Revision validated by the Notified Body
/		/	☐ Yes Validation language: ☐ No (only applicable for class C (IVDR, Article 48 (7)) for which the SSP is not yet validated by the NB)[9]
			☐ Yes Validation language: ☐ No