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TECO®

Aspergillus Galactomannan Assay ELISA Kit

Instructions for use

English

CE

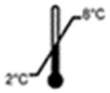
Catalogue No. TE1067
UDI-DI 7640146270078

TE1067_AA-E_01/2025

TECOmedical AG

www.tecomedical.com

Symbol Description

REF *TE1067*	LOT *Lot number*	UDI-DI *Unique Device Identifier*
IVD *In Vitro Diagnostics*	 *Manufacturer*	 *Read electronic kit instructions*
 *96* *96 tests*	 *Expiry date*	 *Storage temperature*
 *Attention*		

Professional Use only

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Any serious incident that has occurred in relation to this product shall be reported to TECOmedical AG and the competent authority of the Member State in which the user and/or the patient is established

TECO® Aspergillus Galactomannan Assay

CONT **Reagents and Materials Supplied:**

SYMBOL	DESCRIPTION	FORMAT
R1	**96-well plate coated with anti-galactomannan antibodies** 12 break apart strips of 8 wells (12 x 8 in total), in a frame Ready to use	**1 plate**
R2	**Negative control Serum** Ready to use	**2 x 1.7 ml**
R3	**Cut-off control serum** Ready to use	**2 x 1.7 ml**
R4	**Positive control serum** Ready to use	**2 x 1.7 ml**
R5	**Conjugate** Ready to use	**1 x 12 ml**
R6 A	**Sample release agent A** Ready to use	**1 x 6 ml**
R6 B	**Sample release agent B** Ready to use	**1 x 6 ml**
R7	**Concentrated Washing Solution 20x**	**1 x 50 ml**
R8	**TMB Substrate Solution** Ready to use	**1 x 12 ml**
R9	**Stop solution - 2M H_2SO_4** 2 M Sulphuric acid Ready to use 	**1 x 8 ml**
	**eIFU indicator** Login address for electronic kit instructions	

Storage

Store kit at 2–8 °C until expiration date.

Intended use

The TECO® Aspergillus Galactomannan test ELISA is used for the detection of Aspergillus Galactomannan antigen in human serum and bronchoalveolar lavage fluid (BAL), offering a diagnostic reference for Aspergillus infection.

Clinical use

With the wide application of broad-spectrum antibiotics, corticosteroid, immunosuppressant, anti-tumor drugs, as well as the prevalence of AIDS and the development of organ transplantation, the invasive fungal diseases (IFD), with a high mortality, is increasing and complicated. The invasive Aspergillosis (IA) is rapidly increasing. The susceptible population is mainly people who receive immunosuppressive therapy, such as hematopoietic stem cell transplantation patients, hematic malignant carcinoma patients, solid organ transplantation patients, bone marrow transplantation patients as well as long-term chemotherapy and corticosteroid therapy patients and severe AIDS patients.

The clinical symptom of IA is non-specific. There are no identical features in CT scan and X-ray. The difficulty of early diagnosis and timely treatment results in a high mortality of 60%~100% [1]. The presence of galactomannan antigen of Aspergillus indicates a prior Aspergillus infection.

Limitations

The TECO® Aspergillus Galactomannan assay is intended to be used as an aid in the diagnosis of Invasive Aspergillosis. Positive results obtained with this assay should be considered in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples and radiographic evidence.

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.

The concomitant use of mold-active anti-fungal therapy in some patients with Invasive Aspergillosis may result in a reduced sensitivity of the TECO® Aspergillus Galactomannan ELISA kit.

The following should be considered about the early Aspargillus 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. However, in some cases, specific factors should be taken into account when interpreting the test:

- 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.
- 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 considered 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.

- 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 ß-lactam treatments should be considered 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.
- 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. Thus, if there is a suspicious positive result in the absence of other clinical signs, it is recommended to investigate the products that the patient is taking and notably their production processes and the origin of the raw materials used.

References

[1] P. Munoz, J. Guinea and E. Bouza.
Update on invasive aspergillosis: clinical and diagnostic aspects
Clin Microbiol Infect 2006, 12 (7) : 24–39.

[2] XIAO Chenlu, Han Lizhong, Ni Yuxing, Guo Xiaokui et al.
Correlation between dynamic monitoring of serum galactomannan antigen and antifungal treatment in adult hematologic patients at risk of invasive aspergillosis.
Chin J Infect Chemother, 1009-7708(2015)04-0364-04.

Assay principle

The TECO® Aspergillus Galactomannan (TE1067) is a sandwich ELISA using galactomannan monoclonal antibody coated plates and anti-galactomannan antibodies with HRP for detection. An antibody - galactomannan - antibody / peroxidase complex is formed in the presence of galactomannan antigen and is followed by a substrate color reaction.

Materials required and not supplied

- Pipettes 100 µl – 1000 µl
- Multichannel pipettes 50 - 300 µl
- Graduated cylinders for diluting reagents
- Polypropylene tubes 0.6 or 1.5 ml, sealed and gas-tight
- Aqua dest or ultrapure water
- Vortex mixer
- Incubator 37°C
- Semi-automatic plate washer (Recommended)
- Microplate reader with 450 nm (reference 620 - 630 nm)
- Software package for data generation and analysis

Warnings and precautions

This kit is intended for in vitro diagnostic use by professional persons only.

Follow the instructions carefully.

Observe expiration dates stated on the labels. Allow the reagents to reach room temperature and use reagents within 30 minutes after opening of the kit.
Refer to" Materials Safety Data Sheet" for more detailed safety information.

Material of animal origin used in the preparation of this kit has been obtained from animals certified as healthy, but these materials should be handled as potentially infectious.

Material of human origin used in the preparation of this kit has been tested and found non-reactive for HIV-1 and HIV-2 as well as for HCV antibodies and HbsAg but should, nonetheless, be handled as potentially infectious

TECOmedical AG is not liable for loss or harm caused by non-observance of the kit instructions.

1. For in vitro diagnostic use.
2. Mix all reagents thoroughly before use
3. Treat all specimen samples and controls provided in the kit as potentially biohazardous material. Follow General Precautions when handling contents of this kit and any patient samples.
4. Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.
5. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
6. Store assay reagents as indicated. The substrate solution should be protected from light and oxidation. When the substrate solution changes from colorless to light blue, the reagent is invalid and should be discarded

7. Do not use coated strips if pouch is punctured.
8. Test each sample in duplicate.
9. Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of liquids.
10.
    a. 2M H_2SO_4 is caustic and can be harmful for skin, eyes, and mucosae.
    b. Handle TMB with care. Do not ingest. Avoid contact with skin, eyes, or clothing. Should there be any contact, wash with water. If ingested, call a physician.
11. A mercury-free preservative is used. Incidental contact with or ingestion of buffer solutions may cause irritation of skin, eyes, or mouth. Should there be any contact, wash with water. If ingested, call a physician.

Preparation of reagents

R1 **96-well plate coated with *anti*-galactomannan antibodies**
12 break apart strips of 8 wells (96 in total) in a frame and sealed in a foil bag.
Fit strip wells firmly into the frame. After opening, return any unused wells to the original foil package and seal.
Store at 2–8°C until expiration date or 8 weeks after opening

R2 **Negative control serum**
Human serum negative for Aspergillus Galactomannan
2 vials of 1.7 ml, ready to use.
Store at 2–8°C until expiration date or 8 weeks after opening

R3 **Cut-off control serum**
Human serum containing Aspergillus Galactomannan
2 vials of 1.7 ml, ready to use.
Store at 2–8°C until expiration date or 8 weeks after opening

R4 **Positive control serum**
Human serum containing Aspergillus Galactomannan
2 vials of 1.7 ml, ready to use
Store at 2–8°C until expiration date or 8 weeks after opening

R5 **Conjugate**
Anti-Aspergillus Galactomannan antibodies conjugated with HRP
1 vial of 12 ml, ready to use.
Store at 2–8°C until expiration date or 8 weeks after opening

R6 A **Sample release agent A**
Alcaline solution
1 vial of 6 ml, ready to use.
Store at 2–8°C until expiration date or 8 weeks after opening

R6 B **Sample release agent B**
Acid Solution
1 vial of 6 ml, ready to use.
Store at 2–8°C until expiration date or 8 weeks after opening

R7 **Wash solution 20x**
1 vial of 50 ml Wash Buffer concentrate. Dilute the 1:20 concentrate with deionized or distilled water up to 1000 ml.
Store undiluted at 2–8°C until expiration date.
The diluted washing solution is stable for 2 weeks at 2–8°C.

R8 **Substrate solution**
1 vial of 12 ml of tetramethylbenzidine. (TMB)
Ready to use. Store at 2–8°C until expiration date or 8 weeks after opening in the dark

R9 **Stop solution - 2M H_2SO_4**
1 vial of 8 ml of 2M Sulphuric acid.
Ready to use. Store at 2–8°C until expiration date or 8 weeks after opening at 2-8°C.

Preparation and stability of samples

Collect samples according to standard laboratory procedures. Avoid cross-contamination among samples. Sample labeling should be clear and correct without mistake.

Sample Type

The assay is validated for serum and BAL fluid, The TECO Aspergillus Galactomannan Assay has not been evaluated for use with plasma or other sample types such as urine or CSF.

1. Serum

Serum samples must be uncontaminated with fungal spores and/or bacteria.

2. BAL fluid

The recommended lavage volume is 100 ml (20 ml each time and repeat 5 times), and the recovery rate is 40% to 60%. For some location with low lavage volume, the recovery rate should be no less than 30%.

BAL fluid samples must be collected in sterile saline and may be tested on neat samples (as is) or supernatants from centrifuged samples (10,000 rpm for 10 min).
BAL fluid samples must be uncontaminated with fungal spores and/or bacteria.

Sample transportation

Sample transportation should comply with national biosafety requirements. Transport and store samples in sealed tubes, unexposed to air.

Stability

1. Serum samples

Maximum 3 days at 2-8°C.

15 months at -20°C or less maximum 5 freeze – thaw cycles.

2. BAL samples

Maximum 3.5 days at 2-8°C

15 months at -20°C or less maximum 5 freeze – thaw cycles.

IMPORTANT NOTE:

FROZEN SERUM OR BAL FLUID SAMPLES STORED IN UNKNOWN CONDITIONS MAY GIVE INACCURATE RESULTS DUE TO CONTAMINATION WITH FUNGI AND/OR BACTERIA.

Sample Handling

Pre-treatment of Serum and BAL samples

All control sera: Negative control serum R2 Cut-off control serum R3 and Positive control serum R4 must be processed at the same time as serum/BAL fluid samples.

1. Pipette 150 µl of serum/BAL and control sera into tubes (2 tubes for the Cut-off Control serum **R3**.
2. Add 50 µl of Sample release agent A **R6 A** into each tube.
3. Vortex the tube for 10 sec.
4. Leave the tubes at room temperature for 10 min.
5. Add 50 µl of Sample release agent B **R6 B** into each tube.
6. Vortex the tube for 10 sec.
7. Leave the tubes at room temperature for 1 min.
8. Collect 100 µl of treated samples for detection.

9. Test the treated samples using the following procedure. After preparation, the treated samples may be collected/saved and stored at 2-8°C for up to 48 hours prior to testing. If analysis of the results indicates that retesting is required, another aliquot of the sample must be treated for testing.

Assay procedure

All determinations (controls and samples) should be assayed preferably as duplicates. When performing the assay, the controls and samples should be pipetted as fast as possible (<15 minutes).
To avoid distortions due to differences in incubation times, the Conjugate, Substrate Solution and Stop Solution should be added to the plate in the same order and with the same time interval as the samples. A multichannel pipette is essential.

Allow all reagents to reach temperature (20–25°C) for at least 30 minutes. During all incubation steps, plates should be sealed with the adhesive foil or a plastic cover. For light protection, incubate in a dark incubator or cover plate with aluminum foil.

1. Allocate the wells of the Microtiter plate **R1** for controls and samples.
2. Pipette 100 µl of treated serum/BAL and negative control, cut-off control and positive control into the corresponding wells.
3. Cover the wells with the adhesive cover and incubate the plate for 60±5 min at 37°C.
4. After incubation, aspirate the wells by using a plate washer, or manually decant by inverting the plate. Wash the wells **3 times** with 300 µl diluted Washing solution per well. After the wash cycle tap the inverted wells on a dry absorbent surface to remove excess wash solution. Use of an automatic plate washer is recommended*.
5. Following the washing step, add 100 µl of conjugate **R5** into each well (multichannel pipette).
6. Cover the microtiter plate with the adhesive cover and incubate it 30±3 mins 37°C.
7. After incubation, aspirate the wells by using a plate washer, or manually decant by inverting the plate. Wash the wells **3 times** with 300 µl diluted Washing solution per well. After the last wash cycle tap the inverted wells on a dry absorbent surface to remove excess wash solution. Use of an automatic plate washer is recommended*.
8. Add 100 µl of the substrate solution **R8** into each well.
9. Incubate the plate in the dark, for 25±2 mins at 37°C
10. Stop the reaction by adding 50 µl of Stop solution **R9** into each well (multichannel pipette). After mixing, measure the color reaction within 5 minutes at 450 nm (reference wavelength between 620 / 630 nm)

***Note:**
For automated plate washers, 5 wash cycles in step 1 and 2 are required.

Calculation of results

The presence of Aspergillus Galactomannan in the sample is determined by the Index Value (I) of each well.
I Value means: the OD of each wells divided by the Average OD value of Cut-off control

$$\text{Index Value Control/Sample} = \frac{\text{Average OD of control or OD of sample}}{\text{Average OD of Cut-off control}}$$

- Negative control Index must be < 0.4
- Positive control Index must be > 1.5

If these criteria are unmet, the test needs to be re-performed.

Interpretation of Results

The following cut off limits were identified in the population studied to obtain the performance characteristics; however, each laboratory may wish to establish their own cut offs values and negative and positive interpretation with their patient population.

1. **Serum Index <0.5 or BAL Index <1.0**

   Serum Index <0.5 or BAL Index <1.0 are considered to be *negative* for Aspergillus Galactomannan antigen.

   Note: A negative result may indicate that the patient's result is below the detectable level of the assay. Negative results do not rule out the diagnosis of Invasive Aspergillosis. Repeat testing is recommended if the result is negative, but the disease is suspected.

2. **Serum Index ≥0.5 or BAL Index ≥1.0**

   Serum Index ≥0.5 or BAL Index ≥1.0 are considered to be *positive* for Aspergillus Galactomannan antigen. For all positive patients, it is recommended that a new aliquot of the same sample (serum/BAL) be repeated.

   Note: An absorbance value of less than 0.000 may indicate a procedural or instrument error which should be evaluated. That result is invalid, and the specimen must be re-run. Regular screening (twice weekly) of serum samples of high-risk patients is recommended to increase the sensitivity and early positivity of the test.

Test performance

Analytical sensitivity

To determine the assay's Limit of Detection (LoD), 4 negative serum and 4 negative BAL samples were spiked with different concentrations of Galactomannan and were measured in 20-fold , in 3 different kit lots.

For serum samples, a LoD of 0.375 ng/ml was determined, for BAL samples the LoD is 0.748 ng/ml.

Diagnostic Sensitivity and Specificity

753 serum samples were measured with the TECO® Aspergillus Galactomannan kit versus a reference method:

Results	Coincidence rate (CI)	95% confidence interval	Number of samples
Positive coincidence rate (Diagnostic sensitivity)	94.11%	91.46 – 96.78%	272
Negative coincidence rate (Diagnostic specificity)	94.80%	93.26 – 96.24%	481

Precision
(Intra assay, N=10 in three different kit lots)

Sample	Mean (l)	SD	CV %
Kit lot 200101	2.02	0.13	6.4%
Kit lot 200102	2.04	0.11	5,2%
Kit lot 200103	2.01	0.088	4.5%

(Inter assay, N=10 in three different kit lots)

Sample	Mean (l)	SD	CV %
Reference sample	2.02	0.107	5.3%

Accuracy

Sample	Coincidence rate
Positive serum (N=30)	93.3%
Negative serum (N = 30)	100%
Positive BAL (N = 30)	90.0%
Negative BAL (N = 30)	86.7%

Interference

Controlled test of potential interfering substances in serum showed that here was no interference in the under-mentioned concentrations.

Substance	Concentration
Hemoglobin	≤ 7 mg/ml
Bilirubin	≤ 300 mg/l
Triglycerides	≤ 7 mmol/l
Rheumatoid factor	>1:80
Anti-nuclear antibody	>1:160
Anti-double stranded DNA antibody	>1:50

Cross reactions

Cross reactant	Number of serum samples	Number of positive values (I value <0.5 - serum)
Candida	25	0
Cryptococcus	18	0
Streptococcus pneumoniae	10	0
Mycoplasma pneumoniae	10	0
Mycobacterium tuberculosis-IgG	10	0
Systemic Lupus Erythematosus	10	0
Rheumatoid Factor	16	0
Mycoplasma pneumoniae-IgM	6	0
Mycobacterium tuberculosis-IgG	10	0
Mycoplasma pneumoniae-IgM and Mycobacterium tuberculosis-IgG	5	0
Cyanobacterium marneffei	3	0
Pneumocystis jirovecii	4	0

TECO® Aspergillus Galactomannan Assay

Assay Procedure – Quick Guide

- Bring samples and reagents to room temperature (20-25°C) for 30 min. Mix the samples well.
- Wash Solution **R7** : Dilute 1:20 with deionized or ultrapure water.
- Pretreat 150 µl of serum/BAL samples and controls **R2 , R3** and **R4** with 50 µl Sample release agent A **R6 A** .
  Vortex for 10 sec, leave at RT for 10 min. Add 50 µl Sample release agent B **R6 B** .
  Vortex for 10 sec, leave at RT for 1 min. Collect 100µl of treated sample for detection.

Prepare the required number of Assay strips **R1**

Pipette 100 µl of treated sample into assay wells.

Incubate for 60 ± 5 min at 37°C.

Aspirate and wash 3 times with 300 µl Washing solution (5 times for automated washing).
Aspirate and tap the inverted wells on a clean dry absorbent surface.

Pipette 100 µl of Conjugate **R5** into each well

Incubate for 30 ± 3 min at 37°C

Aspirate and wash 3 times with 300 µl Washing solution (5 times for automated washing).
Aspirate and tap the inverted wells on a clean dry absorbent surface.

Pipette 100 µl of the Substrate Solution **R8**

Incubate in the dark, for 25 ± 2 min at 37°C

Pipette 50 µl of Stop Solution **R9**

After mixing, read the absorbance value (reference wavelength 620/630nm) at 450nm within 5 minutes.
Calculate the index (I)

Please read Kit instruction before using the Quick Guide