ASPERGILLUS GALACTOMANNAN AG VIRCLIA® MONOTEST

For *in vitro* diagnostic use

VCM073: Sandwich chemiluminescent immunoassay (CLIA) for the qualitative detection of *Aspergillus* galactomannan antigen in serum, plasma and human bronchoalveolar lavage samples. 24 tests.

INTRODUCTION:

Aspergillosis is the name given to all diseases caused by the fungi in the genus Aspergillus and includes allergic, superficial, saprophytic and invasive disease. Invasive aspergillosis (IA) is an important life-threatening infection in immunocompromised patients, especially those with prolonged neutropenia, allogeneic hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT). In immunocompetent hosts, Aspergillus infection is involved in chronic pulmonary aspergillosis (in patients with underlying structural lung disease), allergic bronchopulmonary disease, and allergic sinusitis. The most common species isolated in cases of invasive disease are *A. fumigatus*, followed by *A. flavus*, *A. terreus*, and *A. niger*. *Aspergillus* spp are found in the environment (water, air, soil, decomposing plant matter, household dust, building materials, food). Transmission occurs through inhalation of airborne conidia. Inhaled conidia trigger both innate and adaptive immune responses in immunocompetent receptors.

Mycological diagnosis is challenging, with cultures of bronchoalveolar lavage (BAL) having a low sensitivity. Consequently, fungal biomarkers, such as galactomannan (GM) or beta-D-glucan, and also molecular diagnostic tests have emerged for diagnosing IA. The detection of GM in blood and BAL is a well-established and extensively studied method for the diagnosis of invasive aspergillosis. Serum and BAL GM is recommended for the diagnosis of IA in adult and pediatric in certain patient subpopulations (hematologic malignancy, HSCT), but not for screening in SOT recipients or patients with chronic granulomatous disease. GM is not recommended for routine blood screening in patients receiving mold-active antifungal therapy or prophylaxis, but can be applied to bronchoscopy specimens from those patients. Serial GM measurements can also be used for the follow-up of patients.

GM is a heteropolysaccharide composed of a nonimmunogenic mannan core and immunoreactive galactofuranosyl side chains found in the cell wall primarily of mold-like fungi especially in *Aspergillus spp*. and *Penicillium spp*. but also in other species of fungi. Due to its presence in a diversity of microorganisms, false positive results have been reported in association with administration of certain antibiotics and with other fungal infections, such as *Fusarium spp*. or *Histoplasma capsulatum*. Other sources of false-positives reported in the literature were associated to dietary reactivity, laboratory contamination or fluids used for bronchoalveolar lavage (such as plasmalyte).

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (**C**hemi**L**uminescent **I**mmuno**A**ssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the capture of the antigens in the sample with antibodies adsorbed on the polystyrene surface. Unbound antigen is washed off. Then the antibodies labeled with peroxidase react with the antigen captured, and the unbound conjugate is removed by washing; bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use.
Conjugate is coloured to help in the performance of the technique.
Specific reagents required for the run of the test are included in the kit.

KIT CONTENTS:

1 VIRCLIA® ASPERGILLUS GALACTOMANNAN AG MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:
Wells A, B, C: reaction wells; wells coated with anti-galactomannan monoclonal antibody.
Well D: Conjugate: orange; containing anti-galactomannan monoclonal antibody labelled with peroxidase, and Neolone and Bronidox as preservatives.
Well E: Calibrator: clear; buffered solution containing medium level of galactomannan antigen, and Neolone and Bronidox as preservatives.
Well F: Negative control: clear; buffered solution containing low level of galactomannan antigen, and Neolone and Bronidox as preservatives.
Well G: Substrate component B: clear; containing peroxide.
Well H: Substrate component A: clear; containing luminol.
2 VIRCLIA® SAMPLE SOLUTION GMN: 5 ml of sample treatment solution: clear; phosphate buffer containing EDTA and sodium azide as preservative. Ready to use.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:
-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)
-A CLIA automated processor.
-Precision micropipettes
-Screw cap polypropylene tubes
-Heating block/boiling water bath (see Sample Treatment)
-Centrifuge (See Sample treatment)

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored, closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day
REST OF REAGENTS	Refer to package label for expiration date (at 2-8ºC)

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations. Contamination of the VIRCLIA® SAMPLE SOLUTION GMN may lead to false positive results.
Substrate component A is light sensitive. Avoid light exposure. Substrate solutions should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L., does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For in vitro diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material to minimize the possibility of contamination with *Aspergillus* spores from the environment. Be especially careful when handling samples and liquid reagents since microbial contamination may cause false positive results.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Keep containers for samples and reagents closed while they are not being handled.
6. Do not use in the event of damage to the package.
7. Never pipette by mouth.
8. Reaction wells, conjugate and controls in this kit include substances of animal origin. The kit contains inactivated antigen. Reaction wells are coated with rat monoclonal antibodies. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for waste disposal.
9. Do not use this product in automated processors unless they have been previously validated for that purpose.
10. Sample treatment is essential for the correct performance of the test. Follow the instructions described below and use only heating instruments adequately maintained and calibrated for such purpose.
11. VIRCLIA® SAMPLE SOLUTION GMN contains sodium azide (concentration <0.05%). Avoid contact with acids and heavy metals.

SPECIMEN COLLECTION AND HANDLING:

Serum/Plasma

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen and avoid microbial contamination. Serum/plasma samples are to be refrigerated (2-8ºC) upon collection or frozen (-70ºC) if the test cannot be performed within 5 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

Bronchoalveolar Lavage (BAL)

Collect BAL samples according to standard laboratory procedures. Use of sterile or aseptic techniques will preserve the integrity of the specimen and avoid microbial contamination. BAL samples are to be refrigerated (2-8ºC) upon collection or frozen (-70ºC) if the test cannot be performed within 24 hours. Samples should not be repeatedly frozen and thawed. BAL specimens should be thoroughly mixed prior to testing.

PRELIMINARY PREPARATION OF THE REAGENTS:

All reagents supplied are ready to use.

Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:

SAMPLE TREATMENT

1. Pipette A µl (see Table 1) of each serum/plasma or BAL sample into a 2 ml polypropylene screw cap tube.
2. Add B µl (see Table 1) of VIRCLIA® SAMPLE SOLUTION GMN 2 into each tube and mix thoroughly.

A	B
300 µl	100 µl
390 µl	130 µl
400 µl	133 µl
450 µl	150 µl
500 µl	167 µl
510 µl	170 µl
550 µl	183 µl
600 µl	200 µl
Select volumes in order to be able to retrieve at least 200 µl of supernatant after centrifugation for optimal aspiration in automated instruments.	

Table 1

3. Heat tightly closed tubes for **6 minutes in a heating block at 120°C**. The tube must fit closely inside the hole of the block.

OR

Heat tightly closed tubes for **3 minutes at 100°C in a boiling water bath**.

⚠**Check that the heating block or the water bath has reached the indicated temperature before introducing the tubes.**

4. Carefully remove hot tubes from the block or the water bath and centrifuge at 10000 x g for 10 minutes.
5. Carefully aspirate the supernatant without touching or suspending the pellet and transfer to a new clean test tube.

After preparation, the supernatant may be stored at 2-8°C for up to 48 hours prior to testing. If a second testing is required, another aliquot of the sample must be treated for testing.

- **AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Place the test tube containing the supernatant from the treated sample in the sample tube rack and follow the Operator´s Manual of the Automated Processor.

- **MANUAL**

Contact the manufacturer for further information on the manual procedure.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.

The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:

Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit. The software of the instrument will validate the values obtained for the controls and display them in the results report.

Follow the Operator´s Manual of the Automated Processor. Results cannot be validated if the control values deviate from the expected values.

INTERPRETATION OF RESULTS:
Index= (sample RLU/calibrator RLU)

Interpretation for BAL		Interpretation for serum/plasma	
Index	**Interpretation**	**Index**	**Interpretation**
<0.16	Negative	<0.16	Negative
0.16-0.20	Equivocal	0.16-0.20	Equivocal
>0.20	Positive	>0.20	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with negative results are considered as not containing galactomannan or containing galactomannan levels below the detection threshold of the kit. Repeat testing is recommended if the result is negative, but aspergillosis is suspected.
Samples with positive results are considered as containing galactomannan. In case of a positive result, a new aliquot of the same sample should be retested
Regular screening (twice-weekly) of serum samples of high-risk patients is recommended to increase the sensitivity of the test.

LIMITATIONS:
1. This kit is intended to be used with human serum/plasma or BAL. The performance with other types of samples has not been evaluated.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures, such as microbiological culture, histological examination and radiographic evidence.
4. This test is not intended to replace isolation. This test does not give information on the viability of the microorganism present in the sample.
5. A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected, transported or stored improperly. A negative result does not rule infection. Regular screening is recommended to increase the sensitivity of the test.
6. Positive test results do not rule out co-infections with other pathogens. Microbial contamination of the samples or the reagents may cause false positive results.
7. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely in low prevalence scenarios.
8. The performance of this test has not been evaluated for use in patients without clinical signs and symptoms of infection.
9. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.
10. Positive results in BAL samples from immunocompetent patients should be interpreted with caution.
11. Results close to the cut-off index values should be interpreted cautiously. The use of results from two consecutive positive measurements improves the positive predictive value.
12. Sampling strategies and definitions for positive results may affect the performance of GM assays.
13. GM is not recommended for routine blood screening in patients receiving mold-active antifungal therapy or prophylaxis, but can be applied to bronchoscopy specimens from those patients.
14. False positive GM results have been reported in samples from neonatal population.
15. GM is not recommended for screening in SOT recipients or patients with chronic granulomatous disease.
16. GM or GM cross-reacting epitopes have been found in other genera of fungi (including penicilliosis, fusariosis, histoplasmosis, cryptococcosis and blastomycosis) and in other microorganisms (*Bifidobacterium*).
17. Various sources of false positive reactions have been reported in GM detection assays. These include treatment with antibiotics (piperacillin-tazobactam and amoxicillin-clavulanate), dietary factors or fluids used for bronchoalveolar lavage. In case of a positive result in the absence of other clinical signs, it is recommended an investigation of the products that the patient is taking, paying attention to the origin of the raw materials, especially if fermentation processes with fungal microorganisms were involved.

PERFORMANCES:

- **SENSITIVITY AND SPECIFICITY:**

TEST 1: 290 serum / 23 plasma / 205 bronchoalveolar lavage samples were assayed against a commercial ELISA kit. Indeterminate values were omitted from the final calculations.
The results were as follows:

Samples No.		518
Sensitivity (%)		89
	95% CI	81-94
Specificity (%)		92
	95% CI	86-95
PPV (%)		88
NPV (%)		92
LR+/LR-		-0.98/-0.96

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

TEST 2: 249 serum / 78 bronchoalveolar lavage / 1 pericardial fluid samples were assayed against a commercial ELISA kit.
Indeterminate values were omitted from the final calculations.
The results were as follows:

Samples No.		328
Sensitivity (%)		96
	95% CI	90-99
Specificity (%)		89
	95% CI	83-93
PPV (%)		74
NPV (%)		99
LR+/LR-		-1.09/-1.07

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

- **PRECISION:**

4 samples were assayed. 2 replicates of each one were analyzed in 2 different instruments for 20 days. Within-run precision, between-run precision, between-day precision and between-laboratory precision were determined.

The results were as follows:

Sample	Within-run precision % CV	Between-run precision % CV	Between-day precision % CV	Between-laboratory precision % CV
Calibrator	7.6	5.2	3.3	8.6
Positive sample	4.7	2.1	0.9	5.3
Negative control & Negative sample	No change in the interpretation	No change in the interpretation	No change in the interpretation	No change in the interpretation

CV: Coefficient of variation

- **INTERFERENCES:**

Interferences – ANA/RF:

13 samples known to be positive for antinuclear antibodies and rheumatoid factor were assayed. No interferences with antinuclear antibodies (5 samples tested) were found. No interferences with rheumatoid factor (8 samples tested) were found.

Interferences - Endogenous substances:

3 samples were tested with each interferent. Specifications were fulfilled in all cases. No interferences were found with haemolytic (8.5 g/L hemoglobin), icteric (6 g/L bilirubin), hyperlipemic (4 g/L cholesterol and 11 g/L tributyrin) or hyperproteic (60 g/L ɣ-globulin and 60 g/L albumin) samples.

Interferences – Anticoagulants:

3 samples were tested with each anticoagulant. Specifications were fulfilled in all cases. No interferences were found with heparin (30 UI/mL), citrate (0.3 mol/L) and EDTA (2 mg/mL).

- **CROSS REACTIONS:**

91 samples known to be positive for other microorganisms (cytomegalovirus IgG and IgM, *Mycoplasma pneumoniae* IgG and IgM, *Toxoplasma gondii* IgG and IgM, rubella virus IgG and IgM and *Treponema pallidum* IgG) were assayed.

No cross-reactivity with cytomegalovirus IgG (8 samples tested), *Mycoplasma pneumoniae* IgG (8 samples tested), *Mycoplasma pneumoniae* IgM (7 samples tested), *Toxoplasma gondii* IgG (8 samples tested), *Toxoplasma gondii* IgM (9 samples tested), rubella virus IgG (8 samples tested), rubella virus IgM (5 samples tested) and *Treponema pallidum* (7 samples tested) was found. Cross reactivity with cytomegalovirus IgM (1 out of 6 samples tested) was found.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
Y°C / X°C	Store at x-yºC
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

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