SUMMARY OF SAFETY AND PERFORMANCE (SSP) FungaDia-Aspergillus

1. Introduction

The Summary of Safety and Performance (SSP) is one of the requirements of the new Regulation (IVDR 2017/746), specific for class C and D devices, to enhance transparency and adequate access to information. It intends to provide public access to summarised data on the safety and performance of class C and class D IVD devices to all intended users – professionals and lay persons.

2. Summary of Safety and Performance (SSP)

Requirements based on IVDR Article 29	Potential regulatory sources
Device identification and general information	
Name or trade name including any model number or version	FungaDia-Aspergillus Rapid Test
Manufacturer (name and address)	GaDia SA Route de l'Ile-au-Bois 1A 1870 Monthey Switzerland
Manufacturers single registration number (SRN), if available	CH-MF-000031123
Basic UDI-DI	7649990065ASPMM
Intended purpose of the device	
Intended purpose and indications	FugaDia-Aspergillus is a rapid immunochromatographic test for the qualitative detection of the Aspergillus galactomannan antigen in serum and bronchoalveolar lavage (BAL) fluid from patients suspected of Fungal infections. This test is strictly for medical professional use only and not intended for personal use or home testing. The use of the test and the interpretation of the results should be done by a trained healthcare professional. The result of this test should not be the sole basis for the diagnosis; confirmation testing is required.
Target populations	Adult and pediatric patients with acute or subacute respiratory symptoms or fever or other suspicious symptoms or a known immunocompromised patient. The incidence of Invasive Aspergillosis (IA) in

	immunosuppressed patients is rapidly increasing due to antibiotic abuse.
Contraindications (limitations)	1. The product is only used for the detection of Aspergillus Galactomannan antigen in serum and BAL samples. 2. The test results of this kit are for reference only and should not be used as the only basis for clinical diagnosis and treatment. The clinical management of patients should be comprehensively considered in conjunction with their symptoms, medical history, other laboratory tests and treatment responses.
Device description	
Device description	The principle of the test is colloidal gold immunochromatography. If the sample is positive, the antigens in the sample react with the red-colored nanoparticles and form a complex (Antigen - anti-Aspergillus monoclonal antibodies – gold nanoparticles), which was previously pre-dried on the conjugate pad. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-Aspergillus antibodies present on the membrane (Test line) capture the colored conjugate complex and a red line will appear. If the sample is negative, there is no Aspergillus antigens present or the antigens may be present in a concentration lower than the detection limit. The anti-Aspergillus antibodies present on the membrane (Test line) will not capture the antigen-red-colored conjugate complex (not formed), and the red line will not appear. Whether the sample is positive or not, the nanoparticle complex continues to move across the membrane to the immobilized specific antibodies placed in the control line. The anti-mouse antibodies present on the membrane will react with the anti-Aspergillus antibodies coated on the gold nanoparticles and capture the complex to form a red line. The presence of this

	control red line serves as: (1) verification that sufficient volume is added, (2) that proper flow is obtained and (3) an internal control for the reagents. The control line must always appear.
Reference to previous generation(s) or variants of the device (as applicable) and a description of the differences	N/A
Description of accessories intended to be used in combination with the device (as applicable)	N/A
Description of other devices and products intended to be used in combination with the device (as applicable)	Materials Required but Not Supplied 1. Pipettes and sterile tips 2. Timer 3. Disposable sterile micro-centrifuge tubes (eg. 72.692.005, Sarstedt) 4. Centrifuge 5. Heat incubator
Standards Reference	
Harmonised standards and Common Specifications (CS) applied	IVDD 98/79/EC EN ISO 13485:2016 EN ISO 15223-1:2021 EN ISO 17511:2021 ISO 14971:2019 ISO 18113-1:2009 ISO 18113-2:2009 ISO 20417:2021 ISO 13975:2003 ISO 13612:2002 ISO 23640:2011 ISO 20916:2019 IEC 62366-1:2015+A1:2020
Summary of the Performance Evaluation	
Methods: A retrospective study was conducted at the University Hospital of Grenoble (CHU Grenoble-Alpes, Grenoble, France) using 153 serum samples and 33 samples from Bronchoalveolar lavage (BAL), retrospectively collected at the CHU Grenoble-Alpes in 2021 and 2022. 4 BAL samples (prevalence of 12%) and 49 serum samples (prevalence of 32%) were classified as positive with the CE-IVD PLATELIA ELISA Aspergillus Ag Galactomannan assay (BioRad, Marne-la-Coquette, France), according to manufacturer instruction for use.	

To perform the test, 0.3 ml of defrosted serum sample was diluted in 0.1 ml of treatment buffer, incubated at 130°C for 6 min and centrifuged 10 min at 10'000xg. For BAL samples, 0.3 ml of samples were heated at 100°C for 3 min and centrifuged 10 min at 10'000xg. Then, 50 ul of treated sample were added to the sample hole and incubated at room temperature for 10 minutes. The result was read visually and in parallel with the LFA Reader (Qiagen) to determine the signal intensity (in mV) of test line, blinded to the reference ELISA result. The signal intensity and results of the test were reported and analyzed.

The primary end point was to assess the diagnostic performance of FungaDia-Aspergillus Rapid test (GaDia SA, Monthey, Switzerland) in serum and BAL samples against the ELISA reference method PLATELIATM ELISA Aspergillus Ag Galactomannan assay (BioRad, Marne- la-Coquette, France)

Vassarstats online tool (www.vassarstats.net) was used to calculate sensitivity (SE), specificity (SP), positive and negative predictive values (PPV, NPV), 95% confidence intervals, median, and Interquartile range (IQR); while significance (p-values) was calculated using student t test for independent samples with equal variances. Statistical significance was defined as $p < 0.05$. ROC curve analysis was performed using easyROC (http://www.biosoft.hacettepe.edu.tr/easyROC/). Box plot are generated using BoxPlotR (http://shiny.chemgrid.org/boxplotr/).

Results:

The diagnostic performance of FungaDia-Aspergillus rapid test on serum samples is described below in the Table.

Platelia (BioRad)/Clinical diagnostic

		+	-
FungaDia ICA	+	38	4
	-	11	100
Sensitivity:	77,6%	(CI95%: 63.0-87.8%)	
Specificity:	96,2%	(CI95%: 89.9-98.8%)	
PPV:	90,5%	(CI95%: 76.5-96.9%)	
NPV:	90,1%	(CI95%: 82.6-94.7%)	

Table 2: Diagnostic performance of FungaDia-Aspergillus in Serum samples compared to ELISA Platelia

A total of 11 discordant results showed a negative FungaDia-Aspergillus results while positive with ELISA reference method (false negative results) and 4 false-positive results.

The kappa coefficient was calculated between FungaDia-Aspergillus and reference ELISA method for Serum samples. A kappa coefficient of 0.77 was obtained

The diagnostic performance of FungaDia-Aspergillus rapid test on BAL samples is described below in the Table. Only the results of 30 BAL samples were collected. 1 sample was invalid (no flow, tracheal aspiration sample) and 2 were not tested due to low numer of rapid test kit.

Platelia (BioRad)/Clinical diagnostic

		+	-
FungaDia ICA	+	2	1
	-	0	27

Sensitivity:	100,0%	(CI95%: 20.0-100%)
Specificity:	96,4%	(CI95%: 79.8-99.8%)
PPV:	66,7%	(CI95%: 12.5-98.2%)
NPV:	100,0%	(CI95%: 84.5-100%)

The sensitivity of FungaDia-Aspergillus is good on BAL samples due to low number of samples available and the wrong sample preparation used for the half of samples. These samples were prepared according to BioRad sample preparation.

The specificity of the rapid test is good and similar to Serum samples.

Due to low number of positive BAL samples, the sensitivity of the test need to be interpreted with caution. The kappa coefficient is 0.78 for BAL samples.

Discussion & conclusion:

The main finding of this evaluation study, using an unmatched case control design including 32% (49/153) of positive samples for Serum samples and 12% (4/34) positive samples in BAL positive group, is that the diagnostic accuracy of FungaDia-Aspergillus RDT on serum samples when compared to ELISA Platelia confirmed cases displayed a SE of 78 %, a SP of 96%, a PPV of 91 % and a NPV of 90%, respectively. For BAL samples, due to the low number of positive cases, the performance values need to be evaluated with care. The SE is 100%, a SP of 96 %, a PPV of 67 % and a NPV of 100 % for BAL samples.

Aspergillus galactomannan detection in serum and BAL samples are wiedly used for the diagnosis of Invasive Pulmonary Aspergillosis (IPA). Most of the actual procedure are done in laboratory environment such as ELISA. D'Haese et al (2012) have assessed the clinical validity of such detection. Their conclusions are that detection of GM in BAL fluid samples of patients at risk of IPA has an excellent diagnostic accuracy. Recently, several guidelines have demonstrated the utility of GM detection in BAL fluid. Less data is available for serum samples and the recommendation are for specific cases only.

Gupta et al (2017) have showed that detection of Aspergillus galactomannan in BAL is more sensitive (87.5%) compared to the detection in serum samples (45%). Similarly, other recent studies confirm the observations (Park et al. 2011; Sehgal et al. 2019; Wu et al. 2021).

In order to compare the agreement between FungaDia-Aspergillus rapid test and ELISA, the kappa coefficient was used (Viera & Garrett 2005). When assessing the ability of a test to be helpful to clinicians, it is important that its interpretation is not due to chance. The kappa coefficient gives a valuable information regarding the agreement between 2 observers or tests.

FungaDia-Aspergillus has a kappa coefficient of 0.77 when comparing serum samples with ELISA method and 0.78 for BAL sample. This coefficient is defined as "Moderate agreement" (Viera & Garrett 2005).

There are however some limitations to this study. First, this is the result of a method evaluation study and not a seroprevalence study. Therefore, the PPV obtained here (based

on a 25-30 % prevalence) will be lower in a low prevalence setting. The test was performed in a laboratory environment.

In conclusion, FungaDia-Aspergillus is not meant to replace classical laboratory diagnostic such as ELISA or EIA but could be considered in low-income countries or where laboratory equipment is not available. It could be used as a pre-screening tool to detect Aspergillus galactomannan in Serum and start the appropriate treatment earlier

In conclusion, FungaDia-Aspergillus gives valuable information in only 15 minutes to start the treatment earlier. Perfromance with ELISA methods, longer and requiring laboratory equipment, has moderate agreement with Serum Samples. A larger study with Bal samples should be conducted.

Summary of the Post-Market Performance Follow-Up (PMPF)

Two PMPF were performed in 2022-2023, one at the University Hospital of Besançon (France) and the second one at the University Hospital of Grenoble (France).

1. Prospective clinical evaluation using LBA from patient form Reanimation department of University Hospital of Besançon, France. A total of 100 rapid tests were used for the evaluation. Investigators are Pr Laurence Millon and Dr. Anne Pauline BELLANGER from Parasitology-Mycology Laboratory of CHU Besançon. Results are expected in Q2 2023

Methods:

Results:

Conclusion:

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2. Retrospective & prospective evaluation using LBA and serum from adult patients at CHU Grenoble-Alpes. A total of 125 tests were used.
   Aims of the study are:
   2.1. To evaluate the contribution of the rapid test for the detection in the context of emergency of galactomannan in LBA/serum:
   - in practice: we could at each series of antigen assay, check the positives with the RDT in order to establish a detection threshold (test sensitivity?). *Propose an insightful positioning of the test in the diagnostic tree*

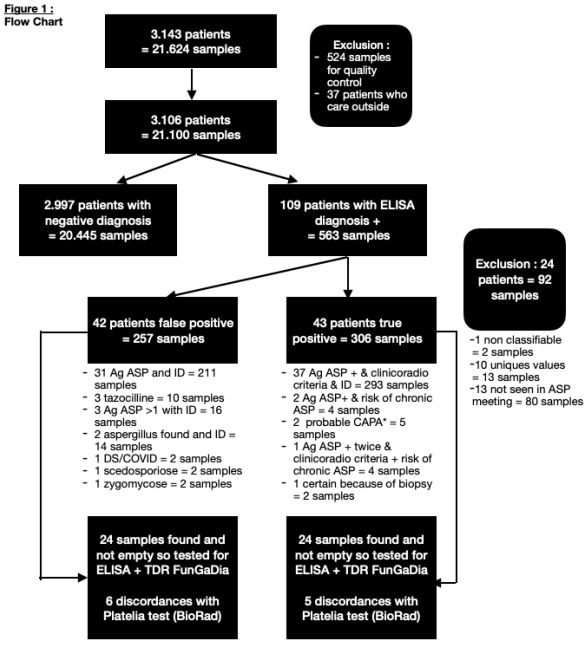
   2.2. Work on the grey areas of the tests: on samples very slightly < 0.5 or very slightly > 0.5 (e.g. between 0.4 and 0.6?) True positives? false negatives? in this problematic we will also extract data on serum passed 'in kinetics' (with one or more priors)
   2.3. Work on cross-reactions (especially interferences with immunoglobulins): in practice: a study of this type has already been done for BDG and platelia. https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(20)30140-3/fulltext#tbl1

Investigators are Pr Muriel Cornet and Dr. Danièle Maubon from Parasitology-Mycology Laboratory of CHU Grenoble-Alpes (France). Results are expected in Q1 2023.

Methods:

The retrospective clinical evaluation was conducted at the University Hospital of Grenoble in December 2022 on 46 characterized and archived serum samples (figure 1). These samples were collected between 2017 and 2022 and included CAPA (COVID-Associated Pulmonary Aspergillosis) patients. Only True positive samples and False positive samples on Platelia were used for this study, in order to exclude any bias in the evaluation. The FungaDia-Aspergillus tests were performed according to the manufacturer's instructions. Prospective samples n=38 (9 BAL and 29 serum samples) were also tested on December 2022 using the rapid test. Sensitivity, specificity, positive and negative predictive values (PPV, NPV) using the Platelia ELISA kit (Bio-Rad) results as reference were calculated.

Figure 1 :
Flow Chart



COVID19 Associated Pulmonary Aspergillosis

Results:

Retrospective study (n=46)

The overall performance of Rapid test kit FungaDia is presented in the figure below. A total of 46 positive samples were used for the retrospective evaluation of the rapid test. The overall sensitivity of the kit is 89%. As no negative samples were evaluated, the specificity was not evaluated in this retrospective study.

Retrospective study

	Platelia	
FungaDia RDT	+	-
+	41	0
-	5	0

Sensitivity:	**89,1%**	(CI95%: 75,6-95.9%)

More negative samples will be tested in the prospective study presented below. The performance of the kit based on the retrospective evaluation is better than the performance tested in January 2022 (sensitivity of 78%)

Prospective study (n=38)

The prospective evaluation was conducted in December 2022, directly after the evaluation with the reference test (Platelia, BioRad) blinded to the reference test. A total of 10 positive samples (6 BAL, 4 serum) and 28 negative samples (3 BAL and 25 serum) were tested. The performance of the kit is presented below.

The sensitivity of the kit was 70% and the specificity 89%. These results must be evaluated with care as only 10 positive samples were evaluated.

Prospective Study

	Platelia	
FungaDia RDT	+	-
+	7	3
-	3	25

Sensitivity:	**70,0%**	(CI95%: 35,4-91.9%)
Specificity:	**89,3%**	(CI95%: 70,6-97.2%)
PPV:	**70,0%**	(CI95%: 35,4-91.9%)
NPV:	**89,3%**	(CI95%: 70,6-97.2%)

Overall performance (n=84)

The overall performance of the kit, combining prospective and retrospective samples (n=84) is presented below on the table. The overall sensitivity is 85.7% and the specificity 89.3%.

	Platelia	
FungaDia RDT	+	-
+	48	3
-	8	25

Sensitivity:	**85,7%**	(CI95%: 73,2-93.2%)
Specificity:	**89,3%**	(CI95%: 70,6-97.2%)
PPV:	**94,1%**	(CI95%: 82,8-98.5%)
NPV:	**75,8%**	(CI95%: 57,4-88.3%)

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FungaDia-Aspergillus

Conclusion:

The performance of rapid test is comparable with similar product on the market with an overall performance (2 studies combined) on serum and BAL samples (n=267) of a sensitivity of 82% and a specificity of 95%.

Metrological traceability	
Metrological traceability of assigned values	N/A
Users	
User Profile	The tests can be performed in laboratories by health care workers or laboratory technicians with appropriate training in sample collection, biosafety and in the use of rapid tests.
User Training	Appropriate training in sample collection, biosafety and in the use of rapid tests.
Device Risks Information	
Residual risks and undesirable effects	- Contamination of the user by infected samples - Wrong interpretation of the test results - False negative - Interference - Cross-reactivity
Warnings and precautions	1. This product is used for in vitro diagnosis, professional use only. 2. Do not reuse the test. Do not use the test after expiry date 3. Please read the test results within the specific time to avoid wrong interpretation. 4. Do not use the components from different batches or different types of reagents. 5. Properly dispose the specimen and used materials following the local biohazardous disposal regulation. 6. Use protective equipment when handling samples and tests as they may contain infectious agents and human or animal components. 7. Sodium azide is used as preservative in the sample treatment solution. Dispose material according to relevant local

	regulations and avoid contact with eyes and skin. 8. When the content of Aspergillus antigen in the sample is very high, the line C may be weakened. 9. Very high concentrations of Aspergillus antigen cause a hook-like effect, leading to false negative results. In this case, it is recommended to use a physiological saline solution to make a 5-10 times dilution of the sample. 10. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established