SUMMARY OF SAFETY AND PERFORMANCE (SSP) FungaDia-Aspergillus ELISA

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 ELISA
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	7649990065ASPEJU
Intended purpose of the device	
Intended purpose and indications	FungaDia-Aspergillus Antigen ELISA is an enzyme sandwich microplate immunoassay Kit for the qualitative detection of Aspergillus galactomannan antigen in adult and pediatric serum samples and bronchoalveolar lavage (BAL) fluid samples of patients with symptoms of, or medical conditions predisposing the patient to, invasive Aspergillus infection in clinical laboratories. The detection of galactomannan in serum or BAL can be used as an aid in the diagnosis of invasive aspergillosis (IA). This ELISA kit should be used in combination with other diagnostic techniques such as microbiological culture, histological examination of biopsies or chest X-ray examination.
Target populations	Adult and pediatric serum samples and bronchoalveolar lavage (BAL) fluid samples of patients with symptoms of, or

	medical conditions predisposing the patient to, invasive Aspergillus infection
Contraindications (limitations)	1. Read the package insert carefully before performing the test. Procedures and the Interpretation of Results must be followed carefully. 2. A negative test from serum and/or BAL samples cannot exclude the diagnosis of Invasive Aspergillosis. Samples from patients at risk for invasive aspergillosis should be tested twice a week or with other diagnostic procedures. 3. A positive result with no clinical signs could be due to the early galactomannan antigen detection in serum or BAL, before the appearance of clinical and/or radiological signs. 4. Cross-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. 5. FungaDia Aspergillus Galactomannan ELISA Detection Kit has not been evaluated for use with plasma or other sample types such as urine or CSF. 6. The results of FungaDia Aspergillus Galactomannan ELISA Detection Kit in Bronchoalveolar Lavage (BAL) samples from non-immunocompromised or neonatal patients should be interpreted with caution. 7. Samples with results close to cut-off index must be interpreted carefully. The clinical management of patients and diagnosis of infectious diseases should be comprehensively considered in conjunction with their symptoms, medical history, other laboratory tests and treatment responses.
Device description	
Device description	FungaDia Aspergillus Galactomannan ELISA Detection Kit is a one-step enzyme sandwich microplate immunoassay which detects galactomannan in human serum and BAL fluid. The assay uses specific

	mouse monoclonal antibody against Aspergillus galactomannan. The monoclonal antibody is used to coat the wells of the microplate and bind the antigen (capture antibody) and to detect the antigen bound to the sensitized microplate (conjugate reagent: peroxidase-linked monoclonal antibody). Serum or BAL fluid samples are heat-treated in the presence of EDTA to dissociate immune complexes and precipitate proteins that could possibly interfere with the test. The treated samples and conjugate are added to the wells coated with monoclonal antibodies and incubated. A monoclonal antibody - galactomannan - monoclonal antibody/peroxidase complex is formed in the presence of galactomannan antigen. The strips are washed to remove any unbound material. Then, the Chromogen TMB solution is added, which will react with the complexes bound to the well to form a blue color reaction. The enzyme reaction is stopped by the addition of an acidic solution, which changes the blue color to yellow. The absorbance (optical density) of specimens and controls is determined with a spectrophotometer at 450 nm.
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)	1. Distilled or deionized water, for dilution of Concentrated Washing Solution (R2) 2. Absorbent paper 3. Protective equipment (disposable gloves and protective glasses) 4. Pipettes or multi-channel pipettes 5. Screw cap micro tubes 1.5 ml (recommended: 72.692.005, Sarstedt) 6. Dry Block Incubator 130°C

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	7. Centrifuge for 1.5 mL polypropylene tubes (10,000 xg) 8. Vortex agitator 9. Microplate incubator at 37 ±1°C 10. Microplate reader equipped with 450 nm filters Note: Automated ELISA Processor (eg. Evolis, BioRad) can be used.
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 and Post-Market Performance Follow-Up

Methods:

A retrospective study was conducted at the University Hospital of Grenoble (CHU Grenoble-Alpes, Grenoble, France) using 203 serum samples and 33 samples from Bronchoalveolar lavage (BAL), retrospectively collected at the CHU Grenoble-Alpes in 2021 and 2022. 5 BAL samples (prevalence of 15%) and 54 serum samples (prevalence of 27%) were classified as positive with the CE-IVD CE-Marked PLATELIATM ELISA Aspergillus Ag Galactomannan assay (BioRad, Marne-la-Coquette, France), according to manufacturer instruction for use.

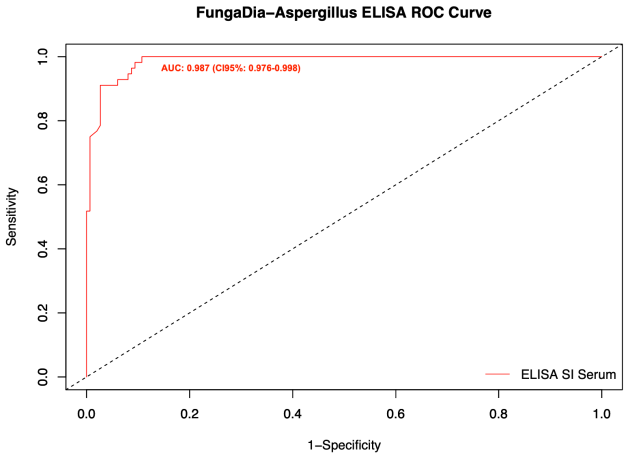
The FungaDia-Aspergillus ELISA kit was performed according to Manufacturer instruction for use. Samples were tested once and the ELISA assay was run on the Evolis automated equipment (Bio-Rad Laboratories)

The primary end point was to assess the diagnostic performance of FungaDia-Aspergillus ELISA (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). A secondary end-point was to assess the impact of automated equipment (Evolis, Bio-Rad Laboratories) on the performance and reproducibility of the test.

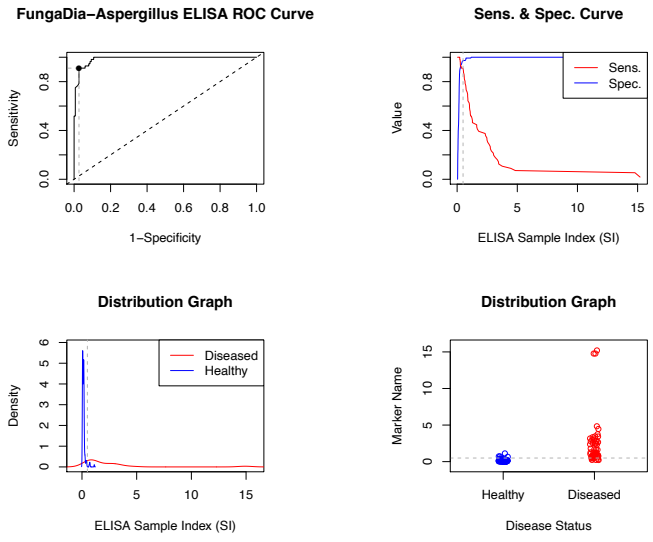
Vassarstats online tool (www.vassarstats.net) was used to calculate sensitivity (SE), specificity (SP), positive and negative predictive values (PPV, NPV), 95% confidence intervals, median, and Interquartile range (IQR); while significance (p-values) was calculated using student t test for independent 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 ELISA on serum samples is indicated in this chapter. The ROC Curve analysis was realized using online software to determine the best cut-off value (Sample Index) and is visible below. The reference method in this chapter is Platelia only



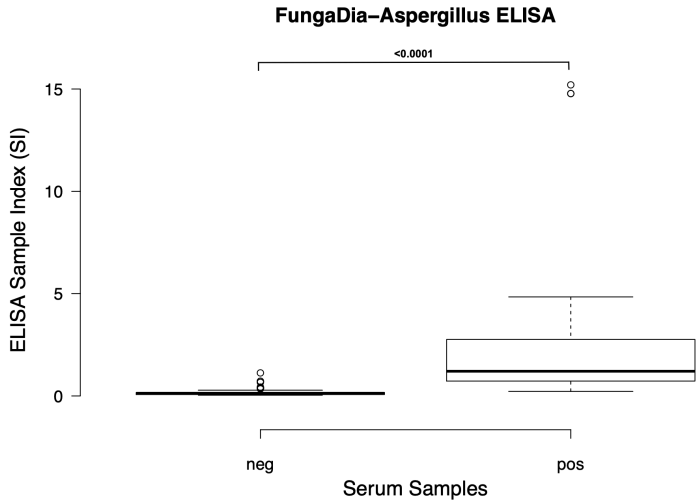
The Area Under the Curve (AUC) is 0.987 (CI95%: 0.976-0.998). The cut-off statistical calculation is presented below in the specific graphs.



The best cut-off value according to Youden Index in a Sample Index (SI) of 0.493.

Therefore, the overall sensitivity of the test is 0.907 (CI95%: 0.804-0.970) and the specificity is 0.993 (CI95%: 0.933-0.993) with this cut-off.

The sample index distribution was also visualized on a Boxplot graph, including the calculation of the p value. The box plot is available below with the statistical analysis.



The diagnostic performance of FungaDia when comparing with reference Platelia is described below in the table.

Platelia (BioRad)

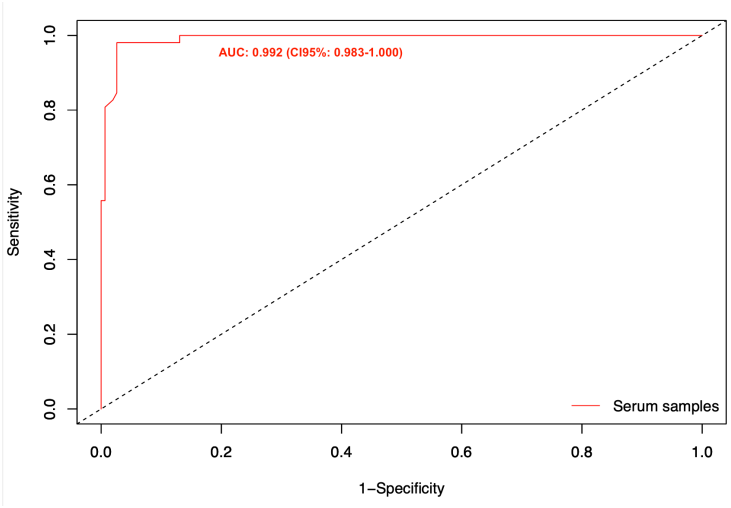
		+	-
FungaDia ELISA	+	49	1
	-	5	148
Sensitivity:	90,7%	(CI95%: 78.9-96.5%)	
Specificity:	99,3%	(CI95%: 95.8-100%)	
PPV:	98,0%	(CI95%: 88.0-99.9%)	
NPV:	96,7%	(CI95%: 92.1-98.8%)	

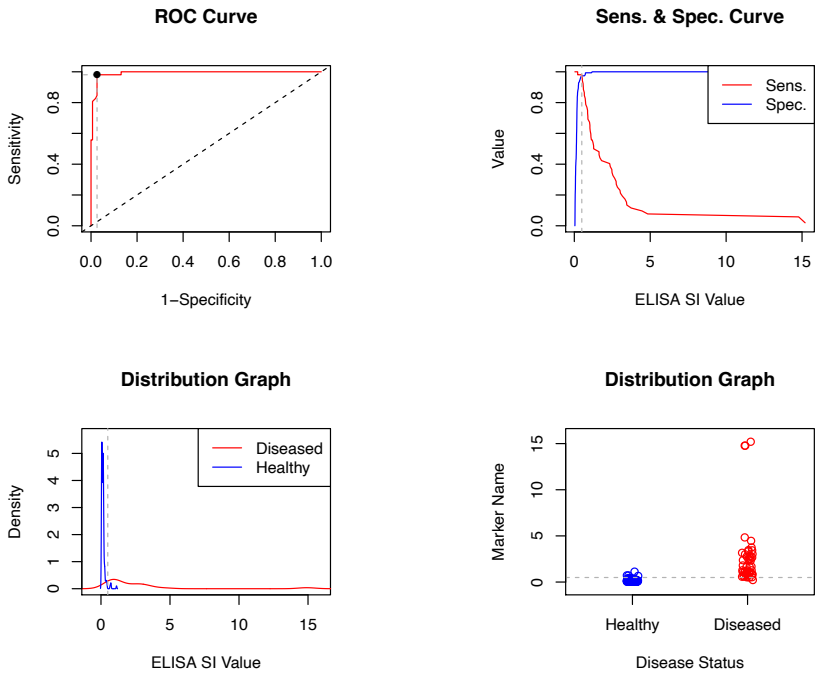
The discordant results obtained with FungaDia-Aspergillus ELISA were reviewed to include the clinical data and history from patients. The false negative results compared to Platelia ELISA (BioRad) were evaluated and four discordant results with FungaDia-Aspergillus ELISA were in fact correct results.

In conclusion, **FungaDia-Aspergillus ELISA has better specificity than ELISA Platelia (BioRad).** If we considered these samples as negative, Platelia has 4 false positive results

out of 153, a specificity of 97% (149/153) and FungaDia-Aspergillus ELISA has a specificity of 99% (152/153).

Corrected ROC curve is presented below:





The corrected performances of FungaDia are presented below. When considering the previous 4 false-negative results, only 1 false negative result is obtained, increasing the sensitivity at 98.1%. The comparison with the Platelia method, when considering also clinical data as reference, confirm the better specificity of FungaDia than Platelia.

Platelia (BioRad)/Clinical diagnostic

		+	-
FungaDia ELISA	+	49	1
	-	1	152
Sensitivity:	98,0%	(CI95%: 87.8-100%)	
Specificity:	99,3%	(CI95%: 95.9-100%)	
PPV:	98,0%	(CI95%: 88.4-99.9%)	
NPV:	99,3%	(CI95%: 95.9-100%)	

The specificity of FungaDia is 99.3% higher thant Platelia (97.4%).

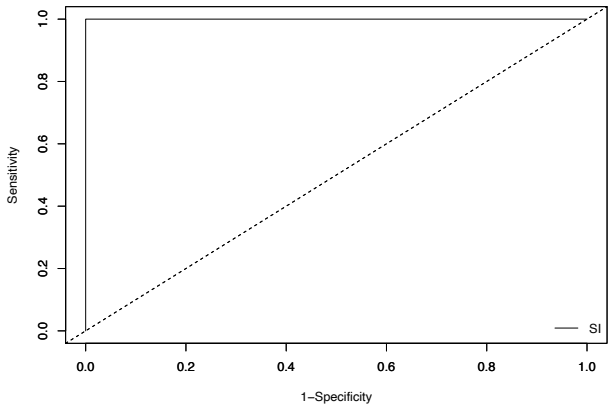
The diagnostic performance of FungaDia-Aspergillus ELISA on BAL samples is indicated in this chapter. The ROC Curve analysis was realized using online software to determine the best cut-off value (Sample Index) and is visible below.

The performance test was conducted on a total of 33 samples.

It is important to highlight that 17 samples were treated according to BioRad procedure and not according to GaDia procedure (sample preparation). The procedure of BioRad includes a dilution in sample treatment and the GaDia procedure has only a thermal treatment, without dilution.

Platelia (BioRad)

		+	-
FungaDia ELISA	+	5	0
	-	0	28
Sensitivity:	100,0%	(CI95%: 46.3-100%)	
Specificity:	100,0%	(CI95%: 85.0-100%)	
PPV:	100,0%	(CI95%: 46.3-100%)	
NPV:	100,0%	(CI95%: 85.0-100%)	



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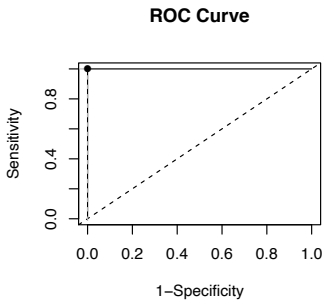
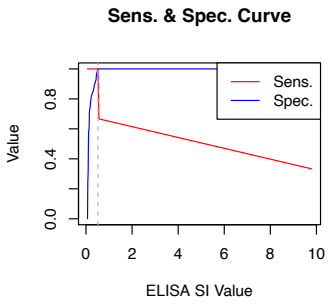
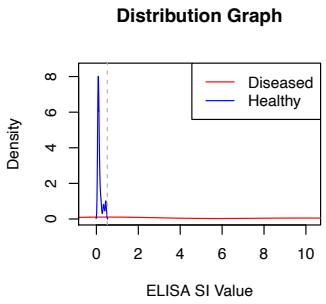
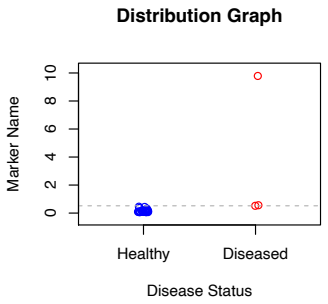
The Area Under the Curve (AUC) is 1.000. The cut-off statistical calculation is presented below in the specific graphs.









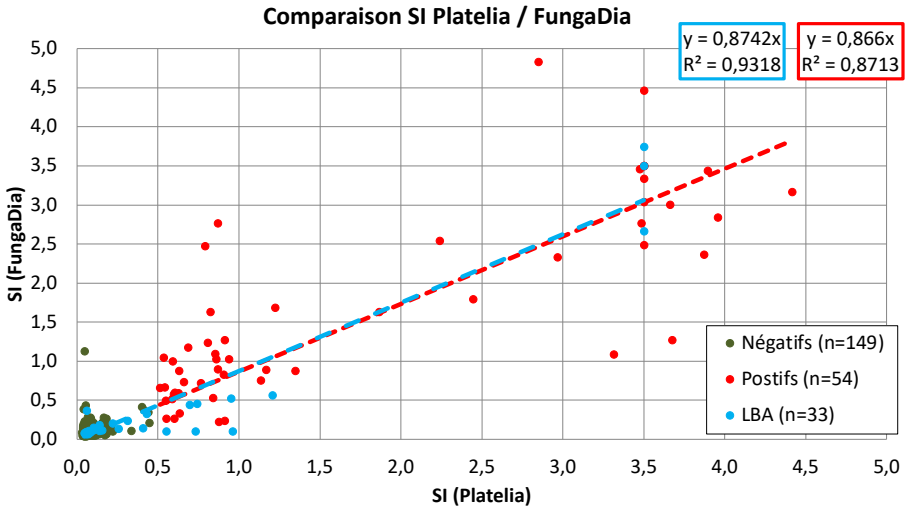
The best cut-off value according to Youden Index in a Sample Index (SI) of 0.52, similar to the serum samples.

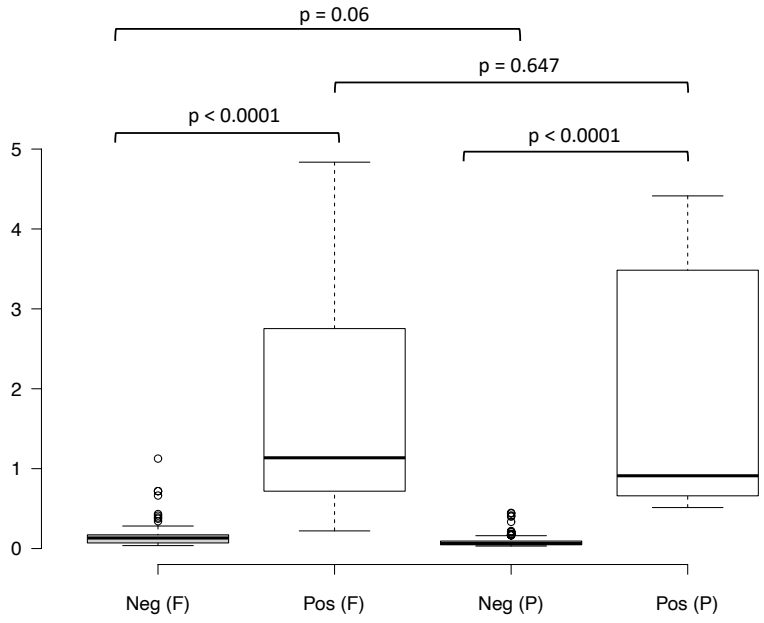
The overall sensitivity of the test is 1.000 (CI95%: 0.292-1.000) and the specificity is 1.000 (CI95%: 0.877-1.000). The large confidence intervale are due to low number of positive samples (n=3).

This evaluation with BAL samples has a low number of positive samples and the results must be considered and interpreted with care.

Data were collected and plotted to compare the samples index from FungaDia-Aspergillus and Platelia ELISA (BioRad) assay to determine if the 2 assays are comparable.

Comparison of SI between Platelia and GaDia (for all samples, serum and BAL) are presented in the 2 graphs below.





Kappa coefficient was calculated to determine the agreement between the 2 methods of analysis. Kappa coefficient was calculated using www.vassarstats.net online statistic.

The kappa coefficient are presented in the tables below. Kappa Unweighted, Kappa with Linear Weighting or Quadratic Weighting were calculated.

The kappa coefficient between the 2 assays is 0.8888 (CI95%: 0.8179-0.9597). According to general kappa table and scientific state of the art, a kappa coefficient of 0.8888 is defined as ***"almost perfect agreement".***

Discussion & conclusion:

The main finding of this retrospective evaluation study, at CHU Grenoble, using an unmatched case control design including 24 % (8/34) of positive samples for BAL samples and 37% (56/205) positive samples in Serum positive group, is that the diagnostic accuracy of FungaDia-Aspergillus ELISA on serum samples when compared to ELISA confirmed cases displayed a SE of 91%, a SP of 97%, a PPV of 93% and a NPV of 97%, respectively and a SE of 100%, a SP of 100%, a PPV of 100% and a NPV of 100% for BAL samples. The low number of positive samples for BAL need to be evaluated carefully.

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. 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).

There are however some limitations to this study. First, this is the result of a retrospective method evaluation study and not a prospective study with high prevalence. 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 at CHU Grenoble on automated equipment (Evolis). The results could differ from manual procedure.

The number of samples for BAL was low, therefore, the sensitivity and CI95% could be impacted.

FungaDia-Aspergillus ELISA has better specificity than ELISA Platelia (BioRad). Platelia has 4 false positive results out of 153, a specificity of 97% (149/153) and FungaDia-Aspergillus ELISA has a specificity of 99% (152/153).

In conclusion, FungaDia-Aspergillus ELISA gives valuable information to start the treatment earlier. Perfromance compared to reference ELISA methods has substancial agreement.

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

A combined prospective and retrospective evaluation was conducted on serum and BAL samples collected at CHU Grenoble Alpes (CHUGA) between 2017 and 2022 using FungaDia-Aspergillus Rapid test and ELISA kit. The primary objective is to confirm the previous results obtained by our team in January 2022. The objectives are also to evaluate if the cut-off of the ELISA kit is good and if there is a grey zone for the ELISA Kit.

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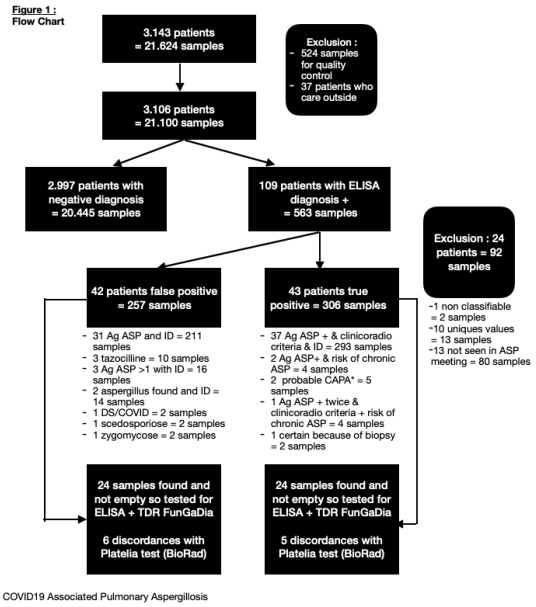
The retrospective clinical evaluation was conducted at the University Hospital of Grenoble in December 2022 on 24 characterized and archived serum samples and 24 BAL samples (figure 1). These samples were collected between 2017 and 2022 and included CAPA (COVID- Associated Pulmonary Aspergillosis) patients. 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. The ELISA FungaDia kit was run on an Evolis (Bio-Rad) automated test system. The threshold of FungaDia-Aspergillus was set at 0.5 for serum and BAL samples, according to the manufacturer's instructions. The threshold of the reference test (Platelia Aspergillus, BIORAD) was 0.5 for serum and 1.0 for BAL. Sensitivity, specificity, positive and negative predictive values (PPV, NPV) using the Platelia ELISA kit (Bio-Rad) results as reference were calculated. In addition, prospectively collected samples were also evaluated with the kit. The graph below presents the total number of samples available at CHU Grenoble Alpes and the exclusion criteria to obtain the final sample size of 48 for retrospective analysis.



In addition to the retrospective samples, prospective samples were collected in December 2022 and analyzed in parallel with the reference Platelia (BioRad).

The table below described the type of sample and the analysis conducted on each sample group.

Sample Group	Collection type	Sample size (Serum – BAL)
I	Retrospective	48 serum (48 pos)
II	Prospective	9 BAL (6 pos / 3 neg); 7 serum (4 pos / 3 neg)
III	Prospective ‘’real’’	51 serum (3 pos / 48 neg)

Group III was a prospective study in real conditions. The samples were analyzed by Platelia (BioRad), blinded, and then tested using FungaDia on Evolis automated equipment (without frozen cycles). This is considered as ''real life'' evaluation.

Results:

1. **Retrospective study**

As this was conducted only with positive samples, only the sensitivity can be calculated.
The result is presented in the table below.

	Platelia
FungaDia	+
+	37
-	11
Sensitivity:	77,1%

Impact of freezing cycle and freezing time is clearly demonstrated during this evaluation and care must be taken when samples are stored.

2. **Prospective study (Group II)**

This first prospective study was conducted on 9 BAL samples and 7 serum samples. The number of samples is very low and the results of this evaluation need to be evaluate with care. In order to increase the number of samples, the study Group I and II will be combined in chapter 4.3.

	Platelia	
FungaDia	+	+
+	7	0
-	3	6
Sensitivity:	70,0%	
Specificity:	100,0%	
PPV:	100,0%	
NPV:	66,7%	

3. **Combination of Retrospective (I) and Prospective (II)**

The performance of **ELISA FungaDia** on all frozen and fresh samples (BAL=9, serum=55) are presented in the table below. The overall sensitivity of the kit is 76% and the specificity is 100%. The sensitivity is higher using BAL samples 83% (5/6) compared to serum samples 75% (39/52).

		Platelia		
		Positif	Négatif	Total
FunGaDia	Positif	44	0	44
	Négatif	14	6	20
	Total	58	6	64

23.4% of samples were discordant when comparing with Platelia. For serum samples, the samples are closed to the cut-off, except for 1 sample and for 1 BAL sample.

4. Prospective study (Group III)

In this prospective study in situ, the samples were directly tested after the reference analysis with Platelia (BioRad) using Evolis, without freezing process. The table below give an overview of the results obtained in this study.

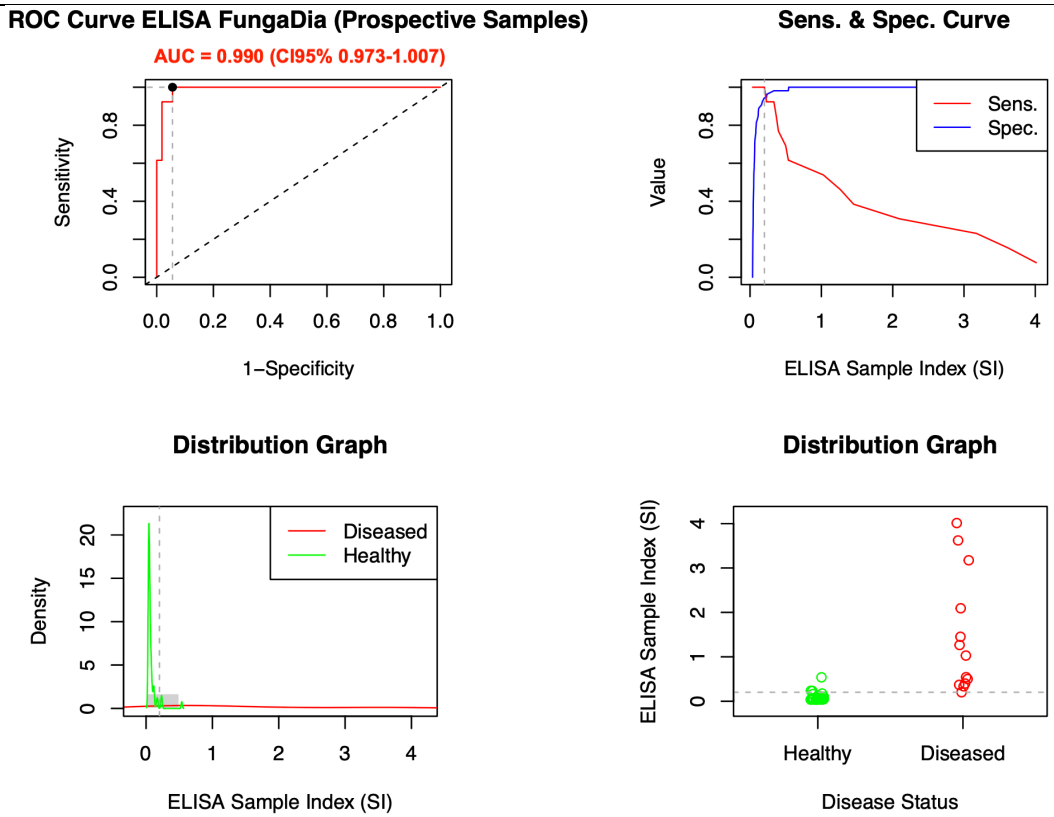
	Platelia	
FungaDia	+	+
+	3	0
-	1	47

Sensitivity:	75,0%
Specificity:	100,0%
PPV:	100,0%
NPV:	97,9%

Only 1 sample was considered negative with FungaDia (SI=0.398) and was positive with Platelia (SI=0.648). One sample was close to the cut-off and was considered positive.

5. Combination of prospective studies (II & III)

The results of the prospectively collected samples (Group II & III), n=67 are presented below with the analysis of ROC curve and determination of best cut-off value (Youden Index calculation).

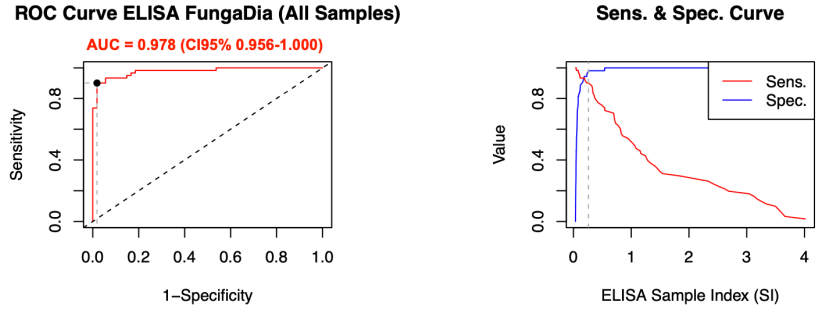


For prospective samples, the best cut-off value was determined at 0.2021, with a sensitivity of 100% and a specificity of 94.4%.

6. Overall performance (I + II + III)

The results of the overall study conducted in December 2022, including 48 retrospective serum samples, 9 prospective BAL samples and 58 prospective serum samples (n=115) are available below.

With a cut-off value of 0.2529, the sensitivity is 90.2% and specificity 98.1%.



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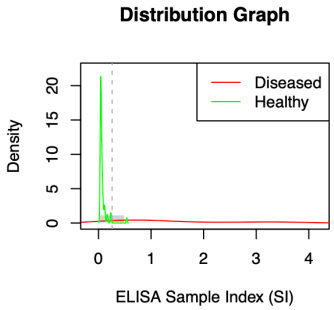

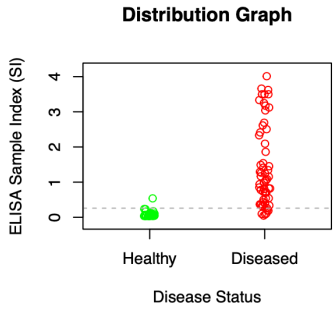

Conclusions

The table below describes the performance of each group of samples and compare the values with the samples collected and tested in January 2022.

Samples	Cut-off	Sensitivity	Specificity
PMPF all (n=115)	0.259	90.2%	98.1%
PMPF prospective (n=67)	0.202	100%	94.4%

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 ELISA kits.
User Training	Appropriate training in sample collection, biosafety, laboratory work and in the use of ELISA kits.
Device Risks Information	
Residual risks and undesirable effects	- Contamination of the user by infected samples or test components - Wrong interpretation of the test results - Cross-contamination between ELISA microwells when performing the test - False negative / false positive - Interference - Cross-reactivity
Warnings and precautions	1. For *in vitro* diagnostic use only. For professional use only, not for self-testing nor near-patient testing. 2. Store frozen serum or BAL fluid samples properly to avoid contamination or degradation of samples.

	3. Do not use kit or kit reagents after expiry date. 4. Do not mix reagents with different lot numbers. 5. Do not re-use microwells. Disassemble the microwell from the plate support with care to not break the microwells. 6. Bring all reagents to room temperature for at least 30 minutes before use. 7. Avoid the formation of bubbles in the wells and mix reagents thoroughly before use 8. Mix thoroughly the Concentrated Washing Solution (R2) before preparing the Working Washing Solution. Crystallization of concentrated washing solution is possible, rinse well the bottle. 9. Use separate and clean pipettes tips for each sample 10. Comply with the recommended number of wash cycles and ensure that all wells are completely filled and then completely emptied. 11. Do not allow the microwells to dry between the end of the wash cycle and addition of reagents. 12. Do not put the Conjugate and Chromogen TMB Solution in the same container. 13. Do not allow Conjugate or Chromogen TMB Solution to come into contact with metal or metallic ions and avoid exposure to strong light 14. Stopping Solution contains acid, avoid contact with eyes and skin. 15. Use protective equipment when using the test and handling samples as they may contain infectious agents, human or animal components. 16. All materials used for this test could contain hazardous substances and human or animal origin components. Refer to national and regional laws and regulations for the disposal of hazardous waste.

	17. Keep testing materials (tubes, tips, containers, etc.) clean, dust-free and sterile to minimize contamination with Aspergillus spores from the environment. 18. The Chromogen TMB Solution must be colorless. The appearance of a blue color indicates the reagent is contaminated and should not be used. 19. 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.