Coordinates of the Curve		
Test Result Variable(s): galactomannan index		
Positive if Greater Than or Equal To*	Sensitivity	1 - Specificity
-.8400	1.000	1.000
.1800	1.000	.953
.2100	.977	.953
.2300	.977	.930
.2550	.977	.907
.2850	.977	.884
.3100	.977	.837
.3250	.977	.767
.3350	.955	.744
.3450	.955	.651
.3550	.955	.628
.3650	.955	.605
.3850	.955	.581
.4100	.955	.512
.4250	.955	.465
.4350	.955	.442
.4450	.932	.419
.4550	.932	.395
.4700	.909	.349
.5100	.909	.326
.5450	.886	.326
.5550	.886	.256
.5650	.864	.256
.6000	.864	.233
.6500	.864	.209
.6950	.841	.163
.7300	.841	.140
.7500	.818	.140
.7700	.795	.116
.7900	.795	.093
.8200	.773	.093
.8500	.773	.070
.8700	.750	.070
.8850	.727	.070
.8950	.705	.070
.9100	.682	.070
.9250	.659	.070
.9400	.636	.070
.9550	.614	.070
.9850	.614	.047
1.0150	.614	.023
1.0450	.591	.023
1.0750	.568	.023
1.1000	.545	.023
1.1600	.523	.023
1.2150	.500	.023
1.3350	.500	.000
1.4450	.477	.000
1.4800	.455	.000
1.5300	.432	.000
1.5550	.409	.000
1.5800	.386	.000
1.6650	.364	.000
1.8150	.341	.000
1.9250	.318	.000
1.9600	.295	.000
2.0250	.273	.000
2.1400	.250	.000
2.2500	.205	.000
2.4200	.159	.000
2.5550	.136	.000
2.8600	.114	.000
3.3250	.091	.000

P420

Fungal detection by means of HCR using 2D-Covalent Organic Framework Nanosheet

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

The timely diagnosis of fungal infections is of prime importance for prescribing appropriate anti-fungal drugs. Current methods for fungal diagnosis involve culture-based methods, antibody-based detection using lateral flow assays and RT-PCR. In the present work, we devised a non-enzymatic amplification using 2D-Covalent Organic Framework (COF) nanosheet for the detection of fungal DNA.

Objectives: (1) Validation of exfoliated 2D COF Nanosheet as an efficient DNA detection tool via Hybridization chain reaction (HCR) triggered fluorescent assay. (2) Sequence retrieval and probe generation of fungal sample and detection of extracted target DNA via fluorescent assay.

Method: A novel COF was synthesized and characterization was done using FTIR, BET, TGA, XRD, and SEM. Probes for the detection of fungi (*Candida*, *Aspergillus*, and *Mucor*) were designed using NUPACK software. HCR was monitored for different time and probe concentrations and standardized reaction was used for the detection of fungal RNA.

Results: FTIR, BET, TGA, XRD, and SEM confirmed the structure and formation of COF-nanosheet. H1, H2 probes at a concentration of 1μM and in presence of Target DNA (0.001μM-1μM) showed HCR reaction at 1.5 h. Fluorescence quenching was observed when probes were mixed with both, bulk COF and COF-nanosheets but increased quenching

Conclusion: Fungal detection can be done by means of HCR using the Covalent nanosheets.

P421

Whole-transcriptome analysis of *Sporothrix brasiliensis* grown in mold- and yeast-inducing conditions

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Objectives: *Sporothrix brasiliensis* is an emerging *Sporothrix* species limited to Brazil capable of causing sporotrichosis in humans and animals, especially in cats. Like other pathogenic *Sporothrix* species, *S. brasiliensis* exhibits a temperature-dependent dimorphic switch and is therefore, able to undergo a reversible morphological transition (mold and yeast), in response to environmental thermal stimuli.

While dimorphism appears to be essential for virulence in *Sporothrix* spp, the molecular mechanisms involved in this phenomenon have not yet been fully elucidated.

In this study, we used the strand-specific RNA-Seq technique and bioinformatics analysis to investigate the transcriptomic signatures associated with mold and yeast phases of *S. brasiliensis*. Furthermore, we generated an accurate version of the *S. brasiliensis* genome annotation in order to perform high-quality gene expression analysis and other functional or structural genomic studies.

Methods: The whole transcriptome of *S. brasiliensis* ATCC-MYA-4823, grown in both yeast-inducing (YPD medium at 37°C) and mold-inducing (YPD medium at 25°C) conditions, was sequenced in this study. High-quality RNA was used to prepare Illumina TruSeq Stranded mRNA-paired-end sequencing libraries (2 × 150 bp) that were sequenced using the HiSeq-2500 platform. A total of three biological replicates were sequenced for each condition.

Before transcriptome assembly, adapters and low-quality reads (Phred-score <25) were removed. The StringTie software was used to assemble the transcriptomes imported into the Apollo webtool to manually curate the genome annotation. Transcripts were investigated using TransDecoder and CPC2 programs to determine whether a gene was potentially protein-coding or non-coding. Finally, differential gene expression analysis between yeast and mold forms of *S. brasiliensis* was conducted using the edgeR package.

Results: Illumina sequencing resulted in a total of ~217 million raw reads. After quality filtering and trimming, ~99.5% of reads were used for downstream bioinformatics analysis. The updated *S. brasiliensis* genome annotation consisted of a total of 14 664 genes of which 10 243 protein-coding genes, 4259 lncRNAs, 140 tRNAs, and 22 rRNAs.

Gene expression analysis revealed a total of 13 838 and 13 938 transcripts expressed in mold- and yeast-form, respectively. Of these, 192 and 292 were expressed exclusively in the mold and yeast-phase, respectively. Moreover, a total of 6802 genes (FDR <0.05) were differentially expressed between the two examined conditions. In particular, 3420 of these genes were up-regulated in the yeast-form (2450 coding, 970 non-coding), and 3382 genes in the filamentous form (2507 coding, 875 non-coding). The raw reads have been deposited into the SRA database and are available under BioProjectID: PRJNA646214.

Conclusions: The characterization of the whole-transcriptome of *S. brasiliensis* mycelial and yeast-like forms represents an essential starting point for investigating the molecular pathways and regulatory frameworks associated with these two morphological stages. Our results provide new insight into global gene expression profiles of *S. brasiliensis*, emphasizing the role of non-coding RNAs in its complex transcriptional network.

All transcriptomic data have also been integrated into the '*Sporothrix* Genome DataBase' (www.sporothrixgenomedatabase.unime.it) in order to expand the current knowledge of *Sporothrix* genomics and to allow a more in-depth structural exploration of *S. brasiliensis* gene models, including gene expression patterns related to its saprophytic and pathogenic lifestyle.

P422

EQUAL PCP Score 2022—an ECMM score derived from current guidelines to measure QUAlity of clinical Pneumocystis Pneumonia management

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Background: Pneumocystis pneumonia (PCP) is a life-threatening opportunistic fungal infection requiring complex clinical management. Guidelines assist clinicians but can be challenging to comply with.

Objectives: To develop a scoring tool, that facilitates and quantifies adherence to guideline recommendations for PCP.

Methods: We reviewed current PCP guidelines and determined essential recommendations for diagnosis, treatment, and follow-up. These were weighted according to their strength of recommendation and level of evidence.

Results: The EQUAL PCP Score 2022 consists of 22 items. For diagnosis, weight was given to bronchoalveolar lavage and immunofluorescence assays as the gold standard for sampling and analysis. Beta-D-glucan was considered of similar importance due to its high negative predictive value. Trimethoprim/sulfamethoxazole and the addition of corticosteroids in respiratory failure got 3 points respectively. Alternative approaches received less points and the use of aerosolized pentamidine was discouraged with 1 minus point. HIV-specific considerations such as the start of secondary prophylaxis were factored in as well.

Conclusion: The EQUAL PCP Score 2022 weighs and aggregates factors recommended for optimal management of PCP. It provides a tool for antifungal stewardship as well as for measuring guideline adherence but remains to be correlated with patient outcomes.