

Supplementary Materials

Homemade fungal culture media

Medium B+

Amounts per 1000 ml medium

Weigh	D(+)<u>Glucose-Monohydrate (Merck 1.08342.1000)</u>	16,70 g
	Yeast extract (Conda lab 1702.00)	30,00 g
	Bacto peptone (BD 211677)	6,80 g
	Bacto Agar (BD 214030)	20,00 g
Measure	Milli-Q water	973,5 ml
Dissolve	Heat until completely resolved	
pH	6.6 (\pm 0.2)	
Antibiotics	Ceftazidime; stock solution 12800 mg/L (final conc 32 mg/L)	2,5 ml
	Chloramphenicol; stock solution 40000 mg/L (final conc 50 mg/L)	1,25 ml
	Colistin; stock solution 8000 mg/L (final conc 24 mg/L)	3,00 ml
	Cotrimoxazole; stock solution 12800 mg/L (final conc 128 mg/L)	5,00 ml
Filling out	21 ml per Petri dish	
Shelf life	3 months at 2-8°C	
Quality control	*See quality control	

Scedosporium selective agar (SceSel+)

Amounts per 1000 ml medium

Weigh	Malt extract (Duchefa M1327.0100)	6.25 g
	Maltose (Sigma 101962921)	6.25 g
	Mono-potassium-phosphate (Sigma 102146710)	1.25 g
	Yeast extract (Conda lab 1702.00)	1.0 g
	Magnesium sulfate 7 H ₂ O (Sigma 1.05886)	0.625 g
	Soy peptone (BD 211906)	0.625 g
	Dichloran * One milliliter of a 0.2% solution in ethanol (Sigma 45435)	2 mg
	Benomyl * Dilute in 5 ml ethanol (Sigma 45339)	6 mg
	Bacto Agar (BD 214030)	20.0 g
Measure	Milli-Q water	986,5 ml
Dissolve	Heat until completely resolved	
Antibiotics	Chloramphenicol; stock solution 40000 mg/L (final conc 100 mg/L)	2,5 ml
	Ciprofloxacin; stock solution 40000 mg/L (final conc 100 mg/L)	2,5 ml
	Streptomycin; stock solution 40000 mg/L (final conc 100 mg/L)	2,5 ml
Filling out	21 ml per Petri dish	
Shelf life	3 months at 2-8°C	
Quality control	*See quality control	

Dichloran-Glycerol Agar (DG18)

Amounts per 1000 ml medium

Weigh	DG18 agar base (Oxoid CM0729)	31,5 g
	Glycerol (Duchefa G1345.1000)	220 g
Measure	Milli-Q water	998 ml
Dissolve	Heat until completely resolved	
pH	5,6 (± 0.2)	
Antibiotics	Gentamicin; stock solution 40000 mg/L (final conc 40 mg/L)	1 ml
	Chloramphenicol; stock solution 40000 mg/L (final conc 50 mg/L)	1,25 ml
Filling out	21 ml per Petri dish	
Shelf life	2 months at 2-8°C	
Quality control	*See quality control	

Stock solution antibiotics

	Amount (purified)	Measure (incl %loss)	Dissolve in	Final concentration (mg/L)
Ceftazidime	128 mg	128 mg	10 ml DMSO	12800
Ciprofloxacin	400 mg	408,2 mg	10 ml 1 M NaOH	40000
Chloramphenicol	400 mg	408,2 mg	10 ml Ethanol	40000
Cotrimoxazol (1:19 ratio)				12800
Trimethoprim	12,8 mg	13 mg		640
Sulfamethoxazol	243,2 mg	243,2 mg	10 ml DMSO	121600
Colistine	80 mg	81,2 mg	10 ml milli-Q	8000
Gentamicin sulfate	400 mg	428 mg	10 ml milli-Q	40000
Streptomycin sulfate	400 mg	422 mg	10 ml milli-Q	40000

Quality control

Fungus	Origin
<i>Candida krusei</i>	ATCC 6258
<i>Trichosporon spp.</i>	UKNeq 9480
<i>Exophiala dermatitidis</i>	CBS 150.90
<i>Aspergillus fumigatus</i>	ATCC 204305
<i>Aspergillus flavus</i>	ATCC 204304
<i>Aspergillus niger</i>	ATCC 10535
<i>Aspergillus terreus</i>	UKNeq 3748/2812
<i>Penicillium spp.</i>	Personal collection*
<i>Rasamsonia argillacea</i>	Personal collection*
<i>Paecilomyces variotti</i>	ATCC 22319
<i>Scedosporium apiospermum</i>	UKNeq 4104/3841
<i>Lomentospora prolificans</i>	CBS 467.74
<i>Lichtheimia ramosa</i>	B64137
<i>Mucor circinelloides</i>	Personal collection*
<i>Cladosporium spp</i>	UKNeq 9836

* Identification confirmed by sequencing.

Table S1. Detection rate and prevalence of molds in sputum samples of CF patients.

	Year 1		Year 2		Year 3		Year 4		Year 3		Year 4		Year 4			
	Detection rate		Prevalence		Detection rate		Prevalence		Detection rate		Prevalence		Detection rate		Prevalence	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
<i>Acremonium</i> spp.	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	2	0.8%	2	2.6%
<i>Alternaria</i> spp.	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	5	1.9%	5	6.5%
<i>Aspergillus flavus</i>	5	1.6%	3	4.0%	8	2.6%	4	4.9%	7	2.8%	5	6.0%	10	3.8%	6	7.8%
<i>Aspergillus fumigatus</i>	98	31.6%	37	49.3%	105	34.7%	42	51.9%	90	35.4%	39	46.4%	146	55.9%	54	70.1%
<i>Aspergillus glaucus</i> group	2	0.6%	2	2.7%	1	0.3%	1	1.2%	1	0.4%	1	1.2%	3	1.1%	3	3.9%
<i>Aspergillus nidulans</i>	1	0.3%	1	1.3%	0	0%	0	0%	0	0%	0	0%	4	1.5%	4	5.2%
<i>Aspergillus niger</i>	1	0.3%	1	1.3%	2	0.7%	2	2.5%	2	0.8%	2	2.4%	8	3.1%	8	10.4%
<i>Aspergillus ochraceus</i>	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.2%	1	0.4%	1	1.3%
<i>Aspergillus</i> spp.	1	0.3%	1	1.3%	3	1.0%	3	3.7%	1	0.4%	1	1.2%	6	2.3%	5	6.5%
<i>Aspergillus versicolor</i>	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.2%	1	0.4%	1	1.3%
<i>Aureobasidium pullulans</i>	1	0.3%	1	1.3%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Basidiomycete	4	1.3%	4	5.3%	1	0.3%	1	1.2%	2	0.8%	1	1.2%	7	2.7%	5	6.5%
<i>Cladosporium</i> spp.	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	6	2.3%	4	5.2%
<i>Colletotrichum</i> spp.	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.3%
<i>Exophiala</i> spp.	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.2%	4	1.5%	2	2.6%
<i>Lomentospora prolificans</i>	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	2	0.8%	1	1.3%
<i>Mucor</i> spp.	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.3%
<i>Paecilomyces</i> spp.	0	0%	0	0%	1	0.3%	1	1.2%	0	0%	0	0%	1	0.4%	1	1.3%
<i>Paecilomyces variotii</i>	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.3%
<i>Penicillium</i> spp.	8	2.6%	7	9.3%	18	5.9%	14	17.3%	18	7.1%	15	17.9%	60	23.0%	36	46.8%
<i>Phoma</i> spp.	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.3%
<i>Purpureocillium lilacinum</i>	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.3%
<i>Rhizomucor pusillus</i>	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	2	0.8%	2	2.6%
<i>Rhizomucor</i> spp.	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.3%
<i>Rhizopus arrhizus</i>	1	0.3%	1	1.3%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
<i>Scedosporium apiospermum</i>	0	0%	0	0%	5	1.7%	1	1.2%	3	1.2%	1	1.2%	1	0.4%	1	1.3%
<i>Scopulariopsis brevicaulis</i>	0	0%	0	0%	1	0.3%	1	1.2%	0	0%	0	0%	0	0%	0	0%
<i>Scopulariopsis</i> spp.	0	0%	0	0%	3	1.0%	1	1.2%	0	0%	0	0%	0	0%	0	0%
Unidentified fungus	0	0%	0	0%	4	1.3%	3	3.7%	5	2.0%	5	6.0%	16	6.1%	14	18.2%
Zygomycete	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0.4%	1	1.3%
Any mold	114	36.8%	47	62.7%	135	44.6%	54	66.7%	115	45.3%	48	57.1%	198	75.9%	68	88.3%

Table S2. Detection rate of molds in sputum samples of CF patients that were included in both the retrospective and prospective cohort.

	Rate of detection	
	Retrospective cohort (n=753)	Prospective cohort (n=257)
Any mold	42.4%	75.5%*
<i>A. fumigatus</i>	35.6%	55.3%*
<i>Aspergillus</i> species	4.1%	12.8%*
<i>Penicillium</i> species	4.8%	23%*
<i>Exophiala</i> species	0.1%	1.6%*
<i>Scedosporium</i> species ^a	0%	1.2%*
Other fungi ^b	2.4%	15.2%*
Any mold excluding <i>A. fumigatus</i>	11%	42.0%*

^a Includes both *Scedosporium apiospermum* and *Lomentospora prolificans*. ^b All fungi that do not group in any of the above. * Significant increase (p<0.05) between the prospective cohort and the retrospective cohort.

Table S3. Time until detection.

	Time until detection					
	Week 1		Week 2		Week 3	
	no. of strains	Percentage	no. of strains	Percentage	no. of strains	Percentage
Time until detection all media						
Any mold	398	74.3%	105	19.6%	33	6.2%
<i>A. fumigatus</i>	259	79.7%	52	16%	14	4.3%
<i>Aspergillus</i> species	28	70%	12	30%	0	0%
<i>Penicillium</i> species	81	73.6%	22	20%	7	6.4%
<i>Exophiala</i> species	2	40%	3	60%	0	0%
<i>Scedosporium</i> species ^a	2	40%	2	40%	1	20%
Basidiomycetes	0	0%	3	37.5%	5	62.5%
Other fungi ^b	26	60.5%	11	25.6%	6	14%
Any mold excluding <i>A. fumigatus</i>	139	65.9%	53	25.1%	19	9%
Time until detection Sabouraud						
Any mold	164	85.4%	24	12.5%	4	2.1%
<i>A. fumigatus</i>	119	91.5%	9	6.9%	2	1.5%
<i>Aspergillus</i> species	11	84.6%	2	15.4%	0	0%
<i>Penicillium</i> species	25	75.8%	7	21.2%	1	3%
<i>Exophiala</i> species	2	100%	0	0%	0	0%
<i>Scedosporium</i> species ^a	1	100%	0	0%	0	0%
Basidiomycetes	0	0%	2	100%	0	0%
Other fungi ^b	6	54.5%	4	36.4%	1	9.1%
Any mold excluding <i>A. fumigatus</i>	45	72.6%	15	24.2%	2	3.2%
Time until detection Medium B+						
Any mold	80	54.8%	50	34.2%	16	11%
<i>A. fumigatus</i>	57	60%	31	32.6%	7	7.4%
<i>Aspergillus</i> species	5	45.5%	6	54.5%	0	0%
<i>Penicillium</i> species	13	52%	9	36%	3	12%
<i>Exophiala</i> species	0	0%	2	100%	0	0%
<i>Scedosporium</i> species ^a	0	0%	0	0%	1	100%
Basidiomycetes	0	0%	0	0%	2	100%
Other fungi ^b	5	50%	2	20%	3	30%
Any mold excluding <i>A. fumigatus</i>	23	45.1%	19	37.3%	9	17.6%
Time until detection Dichloran-Glycerol Agar						
Any mold	149	77.6%	30	15.6%	13	6.8%

<i>A. fumigatus</i>	81	82.7%	12	12.2%	5	5.1%
<i>Aspergillus</i> species	12	75%	4	25%	0	0%
<i>Penicillium</i> species	43	82.7%	6	11.5%	3	5.8%
<i>Exophiala</i> species	0	0%	1	100%	0	0%
<i>Scedosporium</i> species ^a	0	0%	1	100%	0	0%
Basidiomycetes	0	0%	1	25%	3	75%
Other fungi ^b	13	65%	5	25%	2	10%
Any mold excluding <i>A. fumigatus</i>	68	72.3%	18	19.1%	8	8.5%

^a Includes both *Scedosporium apiospermum* and *Lomentospora prolificans*. ^b All fungi that do not group in any of the above.