Millist esmaabi pakub labor aspergilloosi diagnoosimisel?

Projekt „*Aspergillus spp* susceptibility to antifungals in Estonia“

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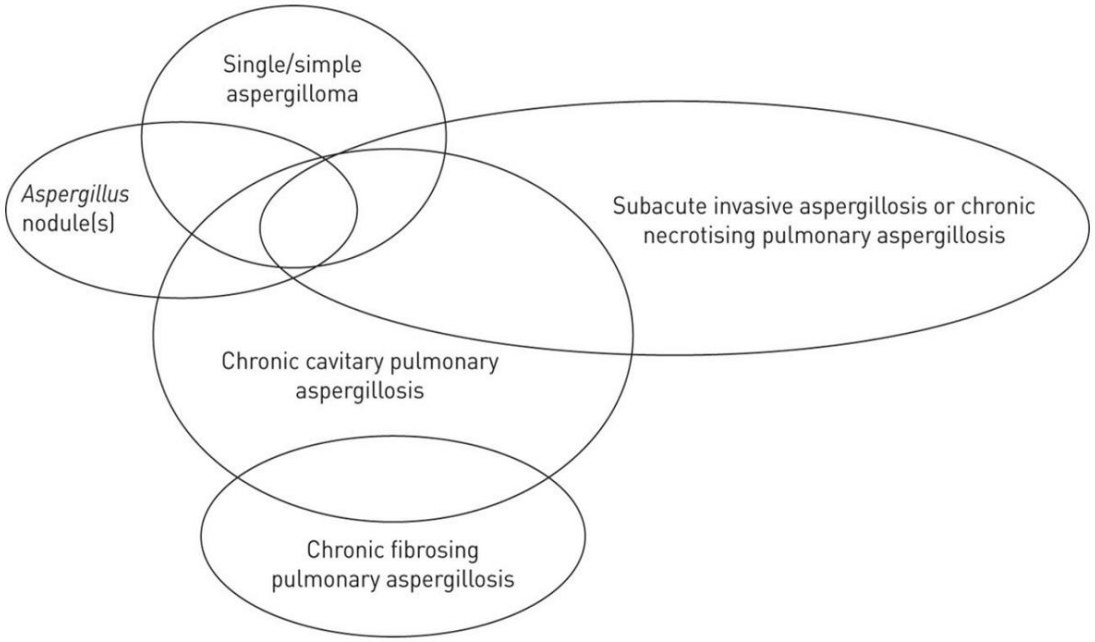
16.09.2020

Aspergilloos – mis haigus see on

- *Aspergillus spp* kolonisatsioon
- Allergiline aspergilloos
- Invasiivne aspergilloos (äge)
- Krooniline kopsude aspergilloos

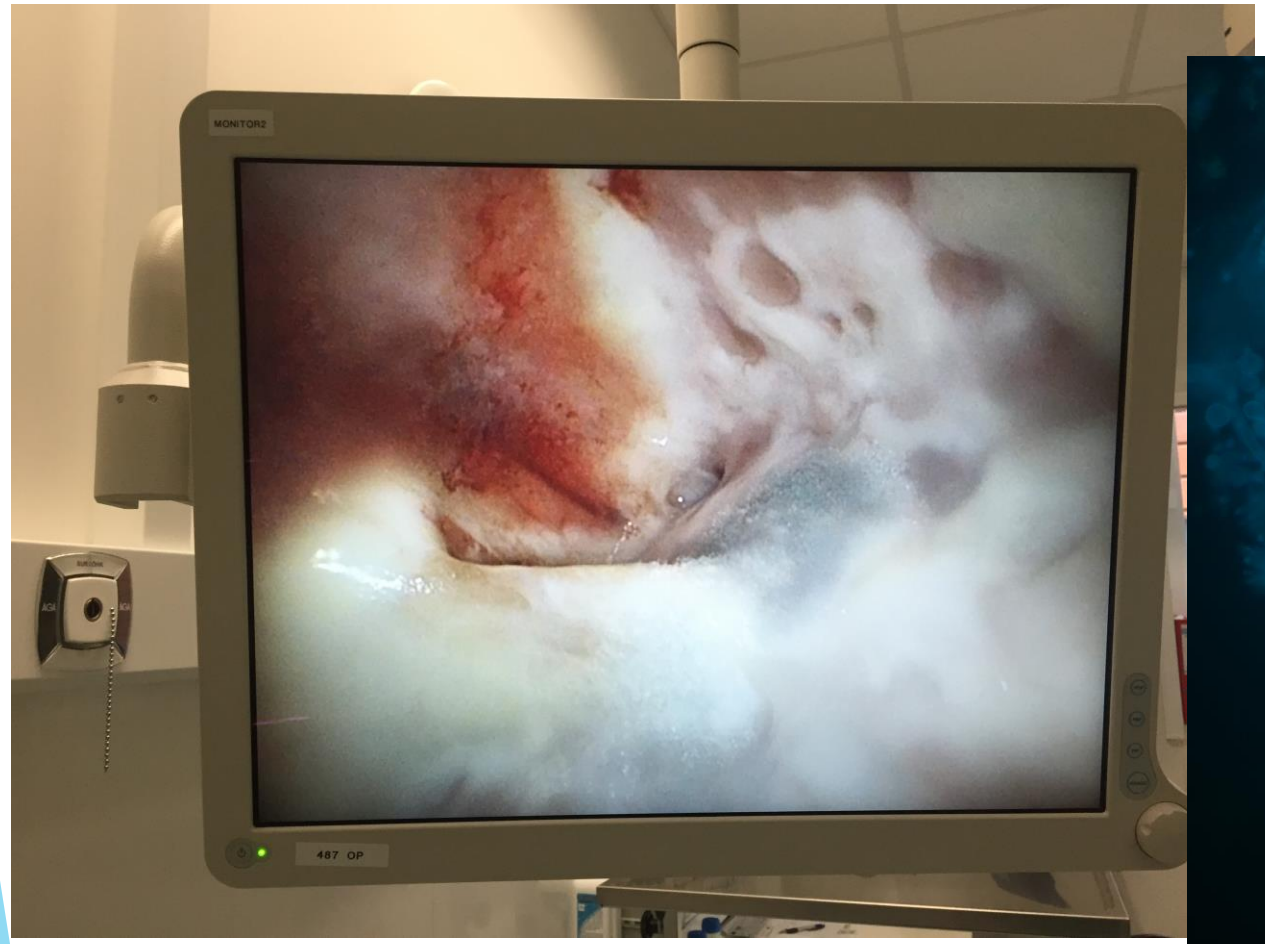


Denning, DW. 2016 Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management Eur Resp Journal
DOI: 10.1183/13993003.00583-2015

Seeninfektsiooni diagnostika
Milline tekitaja, milline biomarker ?
Vaatame ravijuhist

	Pneumocystis	Aspergillus	Candida	Mucor	Cryptococcus
Mannan (+ mannani antikehad)	-	-	+	-	
Galaktomannan	-	+	-	-	-
Polüsahhariidid	-	-	+	-	+
Beeta-glükaan	+	+	+	-	-
Disahhariidid	-	+	+	+	?
Glükoproteiinid (+JF4 antikehad)	-	+	-	-	-
T2 (+magnet resonans)	-	-	+	-	-

Diagnostilised meetodid
culture based versus non-culture based



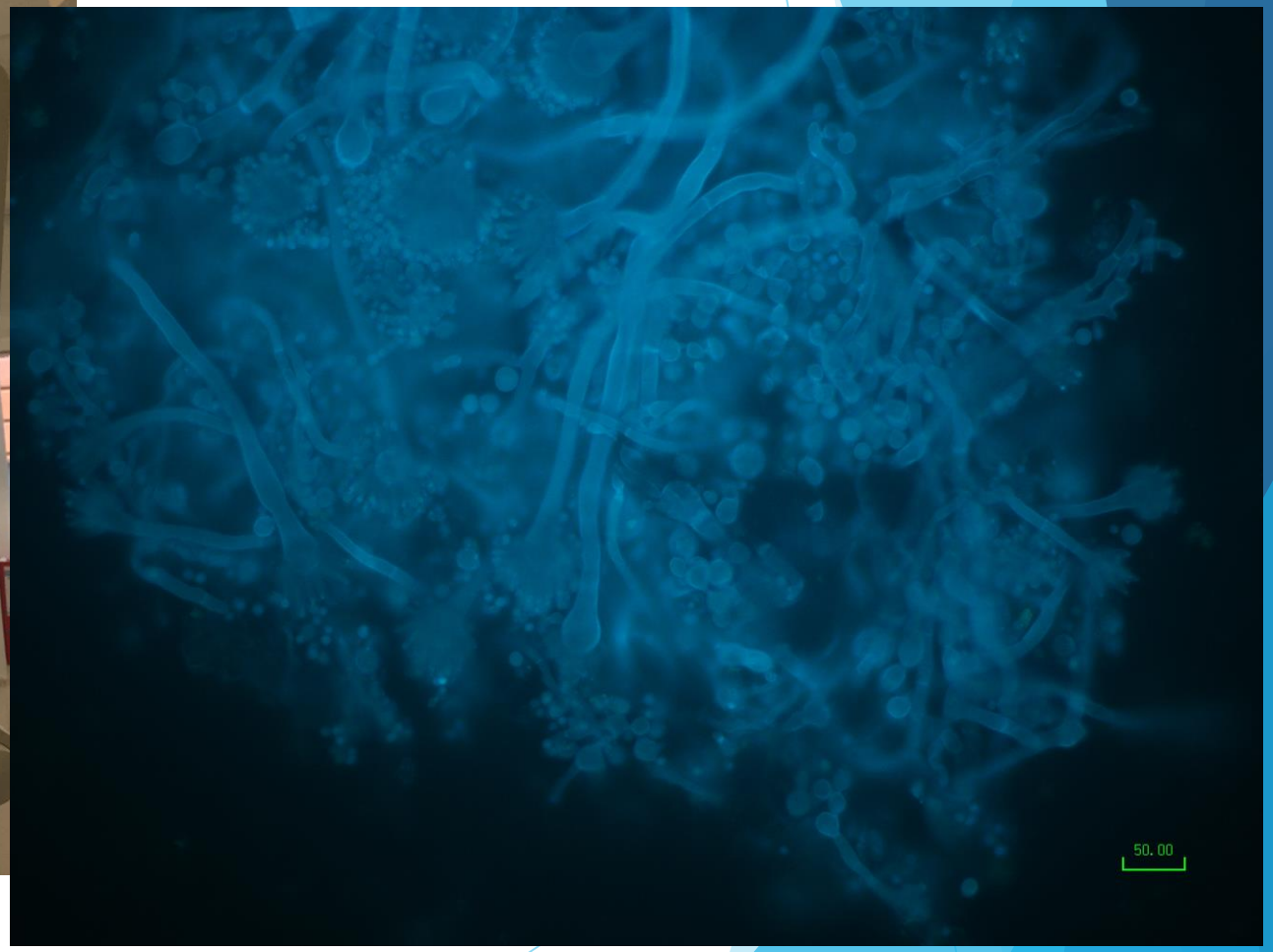


Foto: Dr T Laisaar

Foto: H. Järv

Diagnostilised meetodid
Non-Culture Based

Core Recommendations for Antifungal Stewardship: A Statement of the Mycoses Study Group Education and Research Consortium

Table 1. Comparison of United States Food and Drug Administration–Approved Non-Culture-Based Diagnostic Tests for *Candida* and *Aspergillus*

Parameter	Serum (1→3)-β-D-Glucan (*Candida*) [58–60]	Serum Mannan/ Anti-mannan (*Candida*) [61]	Blood T2Candida (*Candida*) [62, 63]	PCR (*Candida*) [64]	Galactomannan (*Aspergillus*) [65]	Serum (1→3)-β-D-Glucan (*Aspergillus*) [66, 67]
Sensitivity	80%	58%	91%	73%	71%	81%
Specificity	80%	93%	98%	95%	89%	78%
PPV/NPV at 2% prevalence (screening[a])	9%	12.5%	0.5%	16.7%	8%	8%
	>99%	99%	>99%	99%	99%	>99%
PPV/NPV at 10% prevalence (screening[a])	30%	50%	81%	50%	41%	29%
	97%	95%	99%	94%	96%	97%
PPV/NPV at 30% prevalence (diagnosis[b])	<63%	77%	96.4%	74%	72%	62%
	90%	83%	96%	81%	87%	91%

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

[a]Screening: asymptomatic patients without localized signs of infection.

[b]Diagnosis: symptomatic patients with suspected infection.

The Journal of Infectious Diseases® 2020;222(S3):S175–98

Aspergilloosi diagnostika- ja ravijuhis 2 märts 2018

Clinical Microbiology and Infection 24 (2018) e1–e38

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline

A.J. Ullmann [1, 62, 63], J.M. Aguado [2, 62, 63], S. Arikan-Akdagli [3, 62, 63], D.W. Denning [4, 5, 6, 63], A.H. Groll [7, 62, 63], K. Lagrou [8, 62, 63], C. Lass-Flörl [9, 62, 63], R.E. Lewis [10, 62], P. Munoz [11, 12, 13, 62, 63], P.E. Verweij [14, 62, 63], A. Warris [15, 62, 63], F. Ader [16, 17, 65], M. Akova [18, 62, 63], M.C. Arendrup [19, 62, 63], R.A. Barnes [20, 63], C. Beigelman-Aubry [21, 65], S. Blot [22, 23, 65], E. Bouza [11, 12, 13, 62, 63], R.J.M. Brüggemann [24, 62], D. Buchheidt [25, 62, 63], J. Cadranel [26, 65], E. Castagnola [27, 62], A. Chakrabarti [28, 63], M. Cuenca-Estrella [29, 62, 63], G. Dimopoulos [30, 65], J. Fortun [31, 62, 63], J.-P. Gangneux [32, 62, 63], J. Garbino [33, 62, 63], W.J. Heinz [1, 62, 63], R. Herbrecht [34, 62], C.P. Heussel [35, 63], C.C. Kibbler [36, 63], N. Klimko [37, 63], B.J. Kullberg [24, 62, 63], C. Lange [38, 39, 40, 65], T. Lehrnbecher [41, 63], J. Löffler [1, 62, 63], O. Lortholary [42, 62, 63], J. Maertens [43, 62, 63], O. Marchetti [44, 45, 62, 63], J.F. Meis [46, 62, 63], L. Pagano [47, 63], P. Ribaud [48], M. Richardson [4, 5, 6, 62, 63], E. Roilides [49, 50, 62, 63], M. Ruhnke [51, 62, 63], M. Sanguinetti [52, 62, 63], D.C. Sheppard [53, 62, 63], J. Sinkó [54, 62], A. Skiada [55, 62, 63], M.J.G.T. Vehreschild [56, 57, 58, 63], C. Viscoli [59, 62, 63], O.A. Cornely [56, 58, 60, 61, 62, 63, 64, *]

53 autorit
65 institutsiooni

38 lehekülge
38 tabelit -
13 tabelit laboratoorse diagnostika kohta

286 soovitust

Kes koostavad seenhaiguste diagnostika ja ravijuhiseid?

Euroopas

- European Society of Clinical Microbiology and Infectious Diseases (ESCMID)

https://www.escmid.org

ESCMID

MANAGING INFECTIONS PROMOTING SCIENCE

- European Confederation of Medical Mycology (ECMM)

https://www.ecmm.info/

ECMM CEMM

- Nordic Society for Medical mycology (NSMM)

http://www.nsmm.nu/

Nordic Society for Medical Mycology

- Eri riikide meditsiinimükoloogia seltsid (Euroopas 25)

Ameerika Ühendriikides

- Infectious Diseases Society of America (IDSA)

IDSA Infectious Diseases Society of America

ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

AMERICAN SOCIETY FOR MICROBIOLOGY

World Health Organization

Hoenigl, M. et al Global Guidelines and Initiatives from the European Confederation of Medical Mycology to improve Patient Care and Research Worldwide: New Leadership is about Working Together 7 Aug 2018 https://doi.org/10.1111/myc.12836

Klassika – *culture-based*
Külv ja mikroskoopia on endiselt olemas

Table 5
Culture and *Aspergillus* species identification

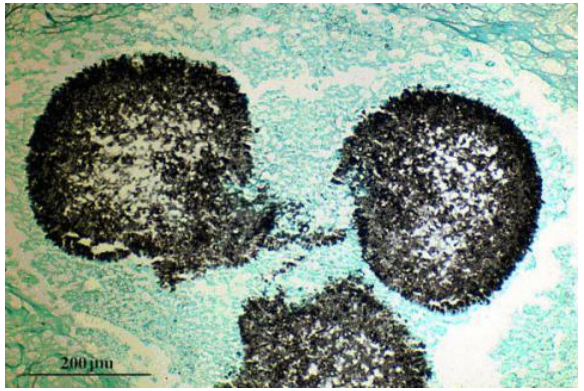
Population	Intention	Intervention	SoR	QoE	Comment	Ref.
Any	Primary isolation from deep sites samples (e.g. biopsies, blood, CSF)	Culture on SDA, BHI agar, PDA at 30°C and 37°C for 72 h	A	III	Blood inhibits conidiation; BHI can help to recover some isolates; isolation of several colonies or isolation of the same fungus from a repeat specimen enhance significance	[81,378,379]
	Primary isolation from non-sterile samples, e.g. sputum, respiratory aspirates	Culture on SDA, BHI agar, PDA with gentamicin plus chloramphenicol at 30°C and 37°C for 72 h	A	III	High-volume sputum culture (entire sample) shown to significantly increase recovery; quantitative cultures are not discriminative for infection or colonization	
	Identification of species complex	Macroscopic and microscopic examination from primary cultures	A	II	Colony colour, conidium size, shape and septation. Colour of conidia and conidiophore and conidiogenesis (tease or tape mounts are preferred); expertise needed for interpretation	
	Identification of species complex (and species identification of *A. fumigatus* specifically)	Culture on identification media at 25–30°C, 37°C and 50°C (2% MEA and Czapek-Dox Agar) and microscopic examination	A	II	Thermotolerance test (growth at 50°C for species confirmation of *A. fumigatus*)	
	Identification at species level	MALDI-TOF MS identification	B	II	In-house databases are often used to improve identification rates	[380–383]
	Identification at species level	Sequencing of ITS, β-tubulin and calmodulin	A	III	Not necessary in organisms with typical growth, but in cases of atypical growth	[384,385]
	To study outbreaks	Microsatellite and CSP analysis	C	II	To study outbreaks (which in general may comprise more than one genotype)	[386–388]
			B	II	To study colonization patterns	[389]

Abbreviations: BHI, brain–heart infusion; CSF, cerebrospinal fluid; CSP, cell surface protein; ITS, internal transcribed spacer; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectometry identification; MEA, malt extract agar; PDA, potato dextrose agar; QoE, Quality of evidence; SDA, Sabouraud dextrose agar; SoR, Strength of recommendation.

Seente mikroskoopia patoloogiakeskustes

- Peamised histoloogias kasutatavad värvimismeetodid HE ning PAS ei ole seente avastamiseks ideaalsed

- Tellida lisaks spetsiifilisemaid värvimismeetodeid (*Grocott methanamine silver*)



- Aspergillus-vastaste antikehadega lisatöötlus (AB nii-öelda üle värvimine)

Laboritel tuleb kriitiliselt üle vaadata proovimaterjali säilitamise ja transpordi soovitused

Table 13
Storage of original samples and isolates

Population	Intention	Intervention	SoR	QoE	Comment	Ref.
Any	To prevent loss of viability of *Aspergillus* in clinical samples, and to reflect the original fungal content	Clinical samples for culture—short-term storage: 4°C to prevent loss of viability and to reflect the original fungal content	A	III		[98,378]
	To prevent degradation of biomarkers, e.g. GM in serum or BALs or bronchial washes	Complete assay soon after delivery to laboratory. Avoid short-term or long-term storage of serum at 4°C	A	I	GM in serum degrades with short-term and long-term storage at 4°C; BAL fluid GM ODI remain stable; testing of pos./neg. serum and BAL fluid pools showed no decline in GM index over 11 months at −20°C	[61,80,369,370,392]
	Short-term maintenance of *Aspergillus* isolates	Repeated sub-culture	A	I	Viability maintained for several years by frequent sub-culture; transfer once a month; maintain at average ambient room temperature	[98,378]
	Long-term preservation of *Aspergillus* isolates	Water storage/storage under mineral oil/silica gel storage/ freeze-drying freezing (−80°C/ ceramic beads/liquid nitrogen)	A	I	Long-term storage means storage periods of 5 years or longer; no further transfers required during this period	

Abbreviations: BAL, bronchoalveolar lavage; GM, galactomannan; ODI, optical density index; QoE, Quality of evidence; SoR, Strength of recommendation.

Milline on patsient, selline biomarker I Aspergilloosi ravijuhis 2018 ja galaktomannan seerumis

Table 6
Galactomannan testing in blood samples

Population	Intention	Intervention	SoR	QoE	Comment	Ref.
Patients with prolonged neutropenia or allogeneic stem cell transplantation recipients **not on mould-active prophylaxis**	Prospective screening for IA	GM in blood[a] Draw samples every 3–4 days	A C	I III	Highest test accuracy requiring two consecutive samples with an ODI ≥0.5 or retesting the same sample Prospective monitoring should be combined with HRCT and clinical evaluation	[82,94,390–394]
Patients with prolonged neutropenic or allogeneic stem cell transplantation recipients **on mould active prophylaxis**	Prospective screening for IA	GM in blood[a]	D	II	Low prevalence of IA in this setting with consequently low PPV of blood GM test Prophylaxis may have a negative impact on sensitivity of the test or the low yield may be due to decreased incidence of IA	[395,396]
Patients with a haematological malignancy	To diagnose IA	GM in blood[a]			Significantly lower sensitivity in non-neutropenic patients	[319,391,397,398]
• Neutropenic patients			A	II		
• Non-neutropenic patients			B	II		
ICU patients	To diagnose IA	GM in blood[a]	C	II	Better performance in neutropenic than in non-neutropenic patients	[89,399]
Solid organ recipients	To diagnose IA	GM in blood[a]	C	II	Low sensitivity, good specificity Most data for lung SOT	[319,400,401]
Any other patient	To diagnose IA	GM in blood[a]	C	II	Piperacillin/tazobactam may no longer be responsible for false-positive results according to recent studies Cross-reactivity in case of histoplasmosis, fusariosis, talaromycosis (formerly: penicilliosis) False-positive results reported due to ingestion of ice-pops, transfusions, antibiotics, Plasmalyt® infusion	[398,402–409]
Cancer patients	To monitor treatment	GM in blood[a]	A	II		[85,353,410]

Abbreviations: GM, galactomannan; IA, invasive aspergillosis; ICU, intensive care unit; ODI, optical density index; PPV, positive predictive value; QoE, Quality of evidence; SoR, Strength of recommendation; SOT, solid organ transplantation.
[a] Serum or plasma.

Milline patsient, selline biomarker II
Aspergilloosi ravijuhis 2018 ja galaktomannan BAL materjalis

Table 7
Galactomannan testing in samples other than blood

Population	Intention	Intervention	SoR	QoE	Comment	Ref.
Any	To diagnose pulmonary IA	To apply GM test on BAL fluid	A	II	GM in BAL is a good tool to diagnose, optimal cut-off to positivity 0.5 to 1.0	[86,88,411–414]
Any	To diagnose cerebral IA	To apply GM test on cerebrospinal fluid	B	II	No validated cut-off	[415,416]
Any	To detect GM in tissue	To apply GM test on lung biopsies	B	II	Using a cut-off 0.5 resulted in a sensitivity of 90 % and a specificity of 95%; specimens need to be sliced, precondition for doing so is that sufficient material is available; dilution in isotonic saline	[61,417]

Abbreviations: BAL, bronchoalveolar lavage; GM, galactomannan; IA, invasive aspergillosis; QoE, Quality of evidence; SoR, Strength of recommendation.

Galaktomannani testimine Eestis

- On pikaajaline kogemus nii Tartus (TÜK Ühendlabor) kui Tallinnas (PERH Diagnostikakliinik)
- Määramine ELISA meetodil
- Vale-negatiivsuse põhjused - seeneravimid, immuun-komplekside moodustumine
- Vale-positiivsuse põhjused – teised hallitused, PTZ, vedelikud (N: tee), bifidobakterid jm

Aspergillus spp uuringuid verest PCR meetodil teostab Eestis 2 laborit

Table 11
PCR on whole blood, serum or plasma

Population	Intention	Intervention	SoR	QoE	Comment	Ref.
Patients with haematological malignancies	To diagnose IA	PCR on blood samples	B	II	Meta-analysis: 16 studies PCR single positive test: Sensitivity: 88%, specificity: 75%; PCR two consecutive positive tests: Sensitivity: 75%, specificity: 87%	[460]
	To diagnose IA	PCR on serum samples			97% of protocols detected threshold of 10 genomes/mL serum volume >0.5 mL, elution volume <100 μL, sensitivity: 86%; specificity: 94%	[461]
	To diagnose IA	PCR on whole blood samples			First blood PCR assay to be compatible with EAPCRI recommendations, fever driven: Sensitivity: 92%, specificity: 95%, negative PCR result to be used to rule out IA	[462]
Haematopoietic stem cell transplantation	To diagnose IA	Prospective screening PCR on whole blood samples	B	II	Combination of serum and whole blood superior	[94–97]
	To diagnose IA	Prospective screening PCR on blood samples	B	II	Addition of GM and PCR monitoring provides greater accuracy, PPV 50%–80%, NPV 80%–90%	[98]
	To diagnose IA	PCR and GM in BAL	A	II		[393]

Abbreviations: BAL, bronchoalveolar lavage; EAPCRI, European *Aspergillus* PCR Initiative; GM, galactomannan; IA, invasive aspergillosis; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; QoE, Quality of evidence; SoR, Strength of recommendation.

Aspergillus spp uuringuid molekulaarsetel meetoditel muudest kliinilistest materjalidest Eestis ei teostata

Table 10
PCR on bronchoalveolar lavage or cerebrospinal fluid

Population	Intention	Intervention	SoR	QoE	Comment	Ref.
Patients undergoing allogeneic stem cell transplantation recipients not on mould-active prophylaxis	To diagnose IA	BAL PCR	B	II		[431]
Patients with pulmonary infiltrates and haematological malignancies and prolonged neutropenia	To diagnose IA	BAL PCR	B	II	Methodically different in-house assays, better performance in patients without antifungal treatment, PCR and galactomannan: increases specificity	[353,411,430,432–452]
ICU patients, mixed populations	To diagnose IA	BAL PCR	B	II	Commercially available *Aspergillus* PCR assays with good performance data	[81,88,450,453–455]
Patients with haematological malignancies	To diagnose CNS aspergillosis or meningitis	CSF PCR	B	II	113 CSF samples from 55 immunocompromised patients sensitivity 100%, specificity 93% (retrospective)	[415,456–459]

Abbreviations: BAL, bronchoalveolar lavage; CNS, central nervous system; CSF, cerebrospinal fluid; IA, invasive aspergillosis; ICU, intensive care unit; QoE, Quality of evidence; SoR, Strength of recommendation.

Table 12
Molecular diagnostics on biopsies

Population	Intention	Intervention	SoR	QoE	Comment	Ref.
Biopsy with visible hyphae	To detect and specify a fungus	Broad-range PCR	A	II	High sensitivity (>90%) and high specificity (99%); various molecular-based techniques available	[61,463]
Biopsy with no visible hyphae	To detect and specify a fungus	Broad-range PCR	C	II	Sensitivity (57%) and specificity (96%); ability to distinguish other fungi; performance only in addition to other tests	[61,463]
Biopsy with visible hyphae	To detect and specify a fungus	Broad-range PCR on wax-embedded specimens	A	II	TaKaRa DEXPAT kit and QIAamp DNA mini kit detected fewer than 10 conidia/sample	[464,465]
Any	To detect and specify a fungus	Fresh tissue samples	B	II	*Aspergillus* PCR performance analysis yielded sensitivity/specificity rates of 86%/100% (79 patients, retrospective study)	[58]

Abbreviations: QoE, Quality of evidence; SoR, Strength of recommendation.

Aspergillus spp antikehade (AB) uuringud

Table 14
Antibody-based diagnosis of invasive aspergillosis [11]

Population	Intention	Intervention	SoR	QoE	Comment	Ref.
Any	To diagnose IA	*Aspergillus*-specific antibodies by EIA: Serion (Germany), Omega (France), Bio-Rad (France), Dynamiker (China)	C	II	Antibodies take a mean of 11 days to develop after onset of illness; detectable in 29% to 100% of patients during course of acute IA	[52,466–472]
		Precipitating antibodies by agar gel double diffusion (Microgen Ltd. UK) or counter-immuno-electrophoresis	C	III	Consider false-negative results due to hypogammaglobulinaemia	[473]
		Agglutinating antibodies by indirect haemagglutination (EliTech/Fumouze, France)	C	II		[473]
		Specific immunoglobulins to *Aspergillus* by ImmunoCap®	C	III		No reference found

Abbreviations: EIA, enzyme immunoassay; IA, invasive aspergillosis; QoE, Quality of evidence; SoR, Strength of recommendation.

- Invasiivse haigusvormi diagnostikas vähetähtis – soovitus CIII
- Eelkõige allergilise aspergilloosi diagnostikaks

(astma + nahatest (*bric skin ja interdermal*) pos + total IgE >1000 ng/mL (416 IU/mL) + Asp IgG & IgE tõus) MedicalMycology, 2017,55,48–55 doi:10.1093/mmy/myw116

- On kasutusel allergoloogia laborites (TÜK, PERH, SYNLAB)
- Pretsipiteeruvad antikehad - Asp IgE ja IgG standardiseerimine puudub

Seeneravimite *Therapeutic drug monitoring* e TDM

Table 3. Sample Care Bundles for Invasive Candidiasis and Invasive Aspergillosis

The Journal of Infectious Diseases® 2020;222(S3):S175–98

Invasive aspergillosis management bundle	
At the time therapy is being started	Serum galactomannan test repeated twice in patients not on mold-active azole prophylaxis
	CT imaging of chest and/or sinus/brain in patients with symptoms localized at these signs
	Early bronchoscopy (within 48 h) with cytology examination and culture of BAL fluid, measurement of galactomannan antigen titer in BAL; transbronchial biopsy if feasible
	Initial appropriate selection and dosing of antifungal agents considering previous antifungal exposure and local epidemiology
	Systematic screening for drug interactions using a computerized drug interactions database for any patient starting or stopping a triazole antifungal agent
After starting therapy	Periodic (eg, weekly) testing of serum galactomannan (if aspergillosis) as an adjunct criterion to assess treatment response
	TDM of voriconazole and posaconazole and possibly isavuconazole serum levels to document adequate drug exposures
	Assessment of therapy appropriateness based on microbiological, culture, or histological results
	Repeat chest CT imaging after 3–4 wk and periodically based on response, to assess infection status and/or progression
	Step-down to oral triazole therapy in patients with a favorable clinical course

Seeneravimite *Therapeutic drug monitoring* e TDM

- Ainus **otsene võimalus** ravi ebaõnnestumise põhjuste ja/või toksilisuse hindamiseks
- Kellele kindlasti? Millisele patsiendile?

Suukaudse ravi korral

Lapsed

Tõsiselt ülekaalulised patsiendid

Kriitilises seisus patsiendid tõsise organpuudulikkusega patsiendid

The Journal of Infectious Diseases® 2020;222(S3):S175–98

Seeneravimite *Therapeutic Drug Monitoring e* TDM võimalused Eestis

- **Vorikonasoolile** TÜK Farmakoloogia instituudis

https://www.kliinikum.ee/yhendlabor/pildid/Dokumendid/tellimislehed/Muud_Saatelehed/Vahendatavate-analyyside-tellimisleht-V19.pdf

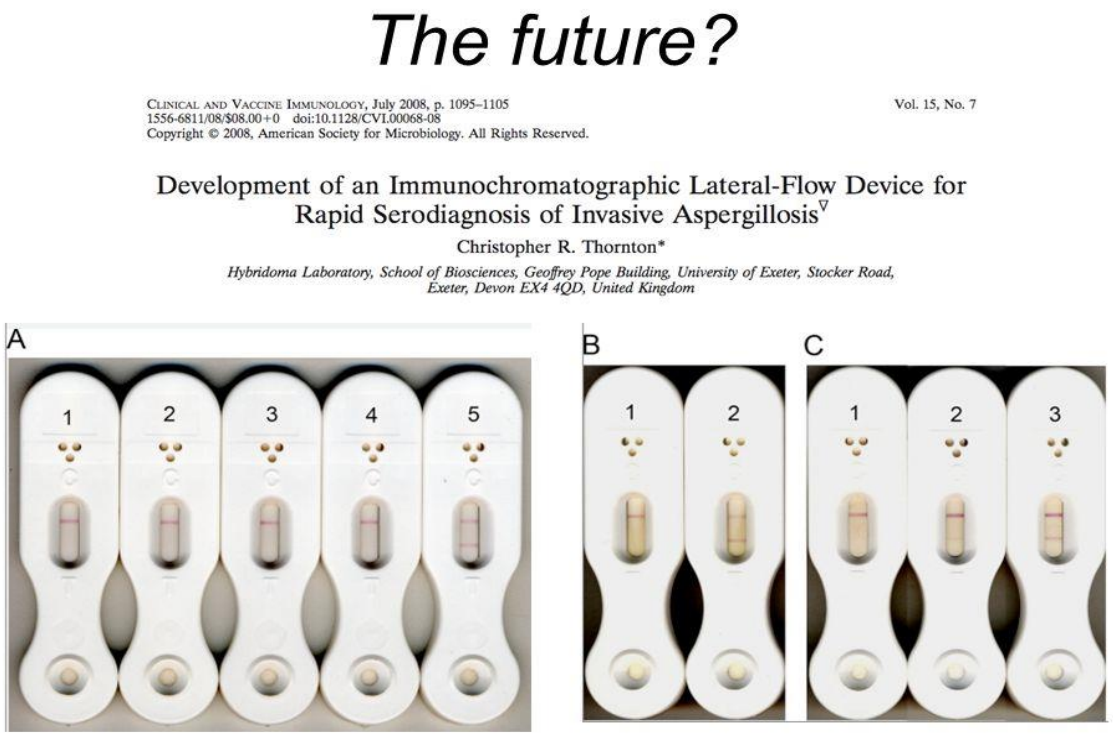
- **Posakonasoolile** TÜK Ühendlaboris; meetod vedelikkromatograafia-massispektromeetria (LC-MS/MS)

https://www.kliinikum.ee/yhendlabor/pildid/kasiraamat/OP/Posakonasool.pdf

- **Itrakonasoolile,** SYNLAB vahendusel Saksamaal, kuid ajaline aken proovivõtu ja vastuse vahel

- **Isavukonaool** ei vaja TDMi

Aspergillus lateral-flow test


The future?

Clinical and Vaccine Immunology, July 2008, p. 1095–1105 Vol. 15, No. 7
1556-6811/08/$08.00+0 doi:10.1128/CVI.00068-08

Development of an Immunochromatographic Lateral-Flow Device for Rapid Serodiagnosis of Invasive Aspergillosis▽

Christopher R. Thornton*
Hybridoma Laboratory, School of Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter, Devon EX4 4QD, United Kingdom

- Point-of-care test
- 15 min

Erinevad testid määravad erinevaid seene komponente

- OLM Diagnostics, UK- JF4 ja glükoproteiini määramine
- IMMY, USA – galaktomannani määramine

- Hindamine silmaga v masinaga (Qiagen aLF reader)

Aspergilloosi ravijuhis & Lateral Flow test

Table 9
Lateral flow device antigen test for invasive aspergillosis

Population	Intention	Intervention	SoR	QoE	Comment	Ref.
Haematological malignancy and solid organ transplant	To diagnose IA	LFD applied on BAL samples	B	II	Retrospective study. Sensitivity and specificity of BAL LFD tests for probable IPA were 100% and 81% (PPV 71%, NPV 100%), five patients with possible IPA had positive LFD, no proven IA	[428]
Haematopoietic stem cell transplantation	To diagnose IA	LFD applied on serum samples	B	II	Prospective screening in 101 patients undergoing allogeneic HSCT	[429]
Immunocompromised patients	To diagnose IA	LFD applied on BAL samples	B	II	Retrospective study. Sensitivities for LFD, GM, BDG and PCR were between 70% and 88%. Combined GM (cut-off >1.0 OD) with LFD increased the sensitivity to 94%, while combined GM (cut-off >1.0 OD) with PCR resulted in 100% sensitivity (specificity for probable/proven IPA 95%–98%).	[430]

Abbreviations: BAL, bronchoalveolar lavage; BDG, β-D-glucan test; GM, galactomannan; HSCT, haematopoietic stem cell transplantation; IA, invasive aspergillosis; IFD, invasive fungal diseases; LFD, lateral device flow; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; QoE, Quality of evidence; SoR, Strength of recommendation.

- On olemas patsiendid
- Uued patsiendirühmad: gripi-seoseline aspergilloos
- Eestis ei ole mingeid takistusi LFD kasutusele võtmiseks

Lateral Flow Device ja BAL ja *Aspergillus*

LFD tootja	Spetsiifilisus	Tundlikkus
Exter (prototype), UK	90%	73%
OLM Diagnostics, UK	100%	71%
IMMY, USA	88%	89%

Thomas F. Patterson and J. Peter Donnelly New Concepts in Diagnostics for Invasive Mycoses: Non-Culture-Based Methodologies J. Fungi 2019, 5, 9, doi:10.3390/jof5010009

C. Lass-Flörl et al. CMI 2019 , 25, 12 https://doi.org/10.1016/j.cmi.2019.08.009 Respiratory specimens and the diagnostic accuracy of Aspergillus lateral flow assays (LFA-IMMY™): real-life data from a multicentre study

Aspergillus Lateral Flow (IMMY ™)

C. Lass-Flörl et al. CMI 2019

- **Methods**

Respiratory specimens (n = 398) from non-selected patients (n = 390) underwent either fungal microscopy, culture or both before Aspergillus lateral flow assay (LFA-IMMY) testing.

- **Results**

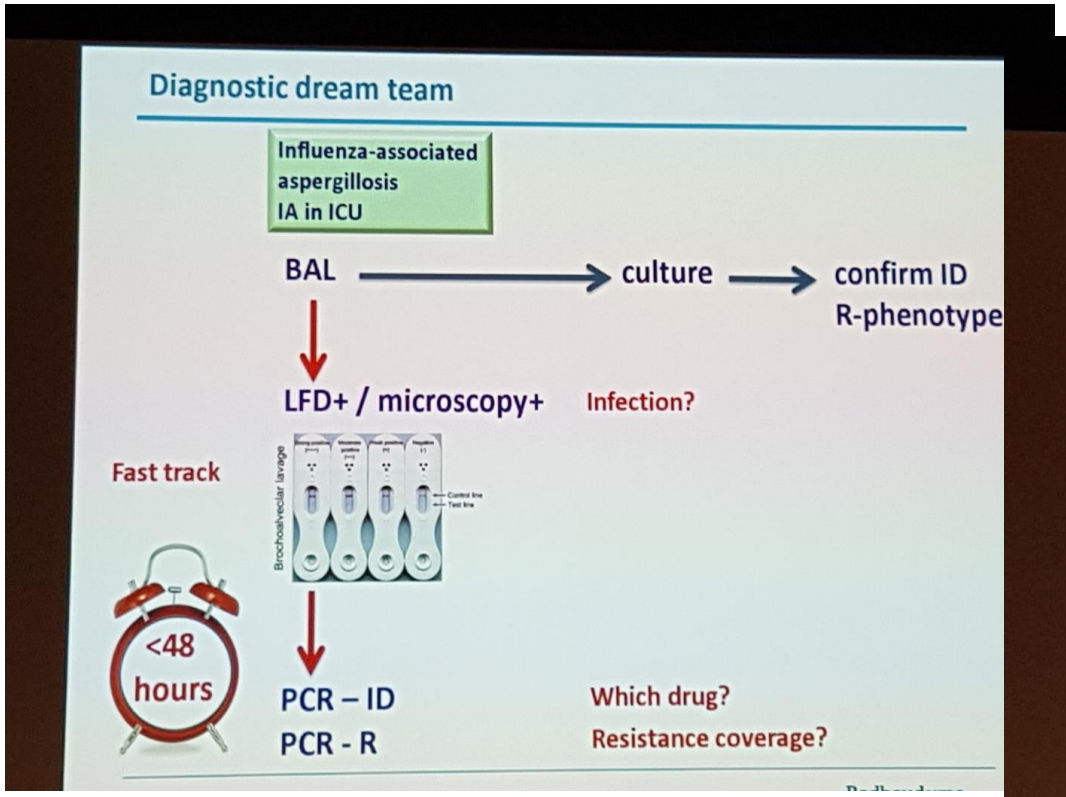
For Aspergillus culture- and microscopy-positive samples, sensitivity (48/52) and specificity (44/48) were 92% (95% CI 8.0%–9.7%) and 91% (95% CI 7.9%–9.7%), respectively; cross-reactivity was documented with non- Aspergillus pathogens.

- **Conclusion**

LFA-IMMY is a reliable diagnostic tool for the detection of Aspergillus in respiratory samples.

Lateral flow device e JF4 ja glükoproteiini määramine

Dr Paul Verweij = Mr Aspergillus ISHAM congress in Amsterdam 2018



Me ei tunne kohalikku epidemioloogiat – Aspergillus spp ravimtundlikkust pole Eestis uuritud

Aspergillus spp resistentsuse andmed

Table 15
Indications for testing for azole resistance in clinical *Aspergillus* isolates

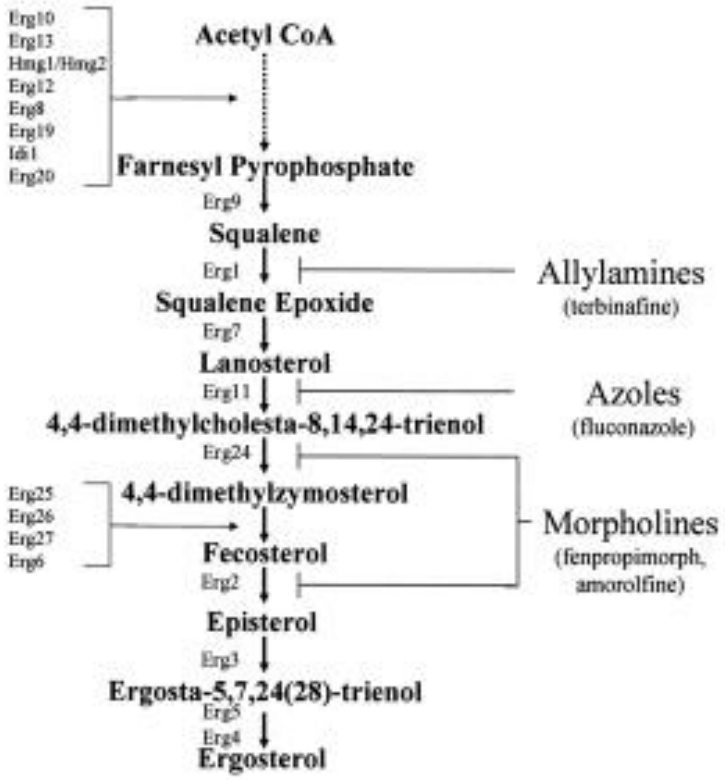
Population	Intention	Intervention	SoR	QoE	Comment	Ref.
All clinically relevant *Aspergillus* isolates (in patient groups or regions with known azole resistance)	Identify azole resistance	Reference MIC testing	A	II	In situations where rapid testing is available	[105,111,114,116,300,474–484]
Clinically relevant *Aspergillus* isolates in patient groups with high prevalence of azole resistance or patients unresponsive to treatment	Identify isolates with intrinsic resistance	Species identification to complex level	A	III	Some species are intrinsically resistant—e.g. *A. calidoustus* (azole resistant) *and A. terreus* (AmB resistant)	[103,485]
Clinically relevant *A. fumigatus* isolates	Identify azole-resistant *A. fumigatus*	Routine azole agar screening	B	III	Identifies resistant colonies that require MIC-testing	[118,486]
All isolates —resistance surveillance	Determine the local epidemiology of azole resistance	Periodical reference MIC testing of *A. fumigatus* complex	A	II	Test at least 100 isolates	[105,111,114,300,477–480,482–484]
Azole-resistant isolates	Determine nature and trends in Cyp51A mutation distribution	Cyp51A-gene mutation analysis	A	II	Test resistant isolates from surveillance survey	[107]

Abbreviations: AmB, Amphotericin B; MIC, minimum inhibitory concentration; QoE, Quality of evidence; SoR, Strength of recommendation.

- Eesti Mükoloogia Uuringutekeskus koostöös SYNLAB, ITKH, TÜ Kliinikum ja PERH on alustanud kliiniliste *Aspergillus fumigatus* tüvede ravimresistentsuse skriining-projektiga Eestis
- Esitame tulemuste kokkuvõte 2020 a lõpul

Resistentsus asoolidele

Linear model of the ergosterol biosynthetic pathway adapted from Saccharomyces cerevisiae.



Chiatogu Onyewu et al. Antimicrob. Agents Chemother.
2003; doi:10.1128/AAC.47.3.956-964.2003

Antimicrobial Agents
and Chemotherapy

Projekt

Aspergillus spp susceptibility to antifungals in Estonia

1. september 2019 - 31. august 2020

Projektis osalejad

Eesti Mükoloogia Uuringutekeskus, ITKH, PERH, TÜK ja SYNLAB

Eesmärk

Uurida Eestis liigi *Aspergillus fumigatus* ravimtundlikkust triasoolide suhtes

Meetodid

Skriiningtesti kasutades määrata vähemalt 100 *Aspergillus fumigatus* ravimtundlikkus 3 asooli itrakonasooli, vorikonasooli ja posakonasooli suhtes

Projekt

Aspergillus spp susceptibility to antifungals in Estonia

Ravimtundlikkuse määramise metoodikast

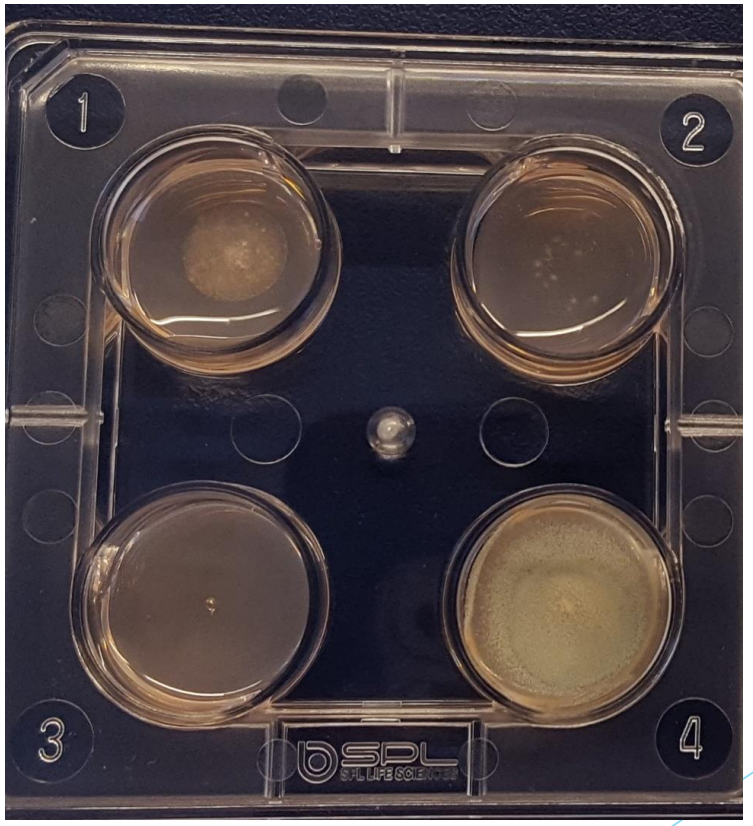
Ullmann, AJ et al. 2018. Diagnosis and managment of Aspergillus diseases: executive summary of 2017 ESCMID-ECCM-ERS guidelines. Clinical Microbiology and Infection 24:e1-e38.

Guinea, J et al. 2019. How to: EUCAST recommendations on the screening procedure E.Def 10.1 for the detection of azole resistance in Aspergillus fumigatus isolates using **four-well azole-containing agar plates**. Clin Microbiol Infect 25 (6), 681-687.

VIP testi kvaliteedikontroll

SSI 4524

SSI 5586



Liigi *Aspergillus fumigatus uuritud* tüved

Kogutud 150 tüve

- 16 Ida-Tallinna Keskhaigla
- 19 TÜ Kliinikum
- 20 Põhja-Eesti Regionaalhaigla
- 20 SYNLAB Eesti

Tüved pärinevad erinevatest kliinilistest materjalidest - 29 kõrv, 22 ülemised hingamisteed, 13 alumised hingamisteed, 2 biopsiat, 1 seerum, 1 pleuravedelik, 1 küünematerjal, 4 määratlemata materjalist

Tulemused

- Kõik uuritud 150 tüve olid **tundlikud** nii itrakonasooli, vorikonasooli kui posakonasooli suhtes

- Kas vajame kliinilise mikrobioloogia laborites rutiinset testimist?

Hetkel, *anno* 2020 ei vaja, andmed olemas

Edasine tegutsemine

- Peame jätkama aeg-ajalt skriining-projektidega - kui tihti???
- Uurida mitte ainult kliinilisi tüvesid, vaid ka põllumajandussaadustelt, õhust isoleeritud *Aspergillus spp* ravimtundlikkust
- Lähimad maad kus resistentsust on leitud, on Poola ja Rootsi
- Tegevus vajaks riiklikku koordineerimist