

Creation of a Biospecimen Registry for the Study and Isolation of Potential Biomarkers Predictive of Progressive Lung Disease in Cystic Fibrosis

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CF-AIR Airways Disease Workshop
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Agenda

- General Overview of the Cystic Fibrosis Biospecimen Registry (CFBR)
- Descriptive analysis of patients who have donated samples to the CFBR and the types of samples currently in the registry
- CFBR utilization by the scientific community
- Current focus on longitudinal samples surrounding acute pulmonary exacerbations (APEs)
- Limitations
- Summary



Cystic Fibrosis Biospecimen Registry (CFBR)

- Principal Investigator: Nael A. McCarty, PhD
- Co-Investigators: Arlene Stecenko, MD
Seth Walker, MD, FCCP
Julie Sedor, MD
Kevin Kirchner, MD
Rabindra Tirouvanziam, PhD
Monal Shah, MD
- Sponsor: Emory University/Children's Healthcare of Atlanta
Center for Cystic Fibrosis and Airways Disease Research



Inclusion/Exclusion Criteria

INCLUSION CRITERIA

- Clinical diagnosis of cystic fibrosis, primary ciliary dyskinesia, bronchiectasis, or CFTR-related metabolic syndrome (CRMS).
- Currently a patient at any of the adult or pediatric CF clinics (no minimum age requirement) at Emory University or Children's Healthcare of Atlanta.
- The subject is able to understand and comply with protocol requirements, instructions and protocol-stated restrictions.

EXCLUSION CRITERIA

- Any condition that, in the opinion of the attending physician, would place the patient at undue risk by participating.
- Known history of HIV, Hepatitis B surface antigen or Hepatitis C antibody.
- Unwillingness or inability to follow the procedures outlined in the protocol.



Study Methods

1. Identify eligible patients attending the adult or pediatric CF clinics at Emory or CHOA
2. Approach patients in clinic, explain the study, and consent if willing to participate
 - Patients fill out consent, assent (if <18 years), and HIPAA authorization
3. Ask participating patients each time they are in clinic or the hospital if they want to donate samples



Objectives

- **Objective 1:** To generate a bank of well-characterized biological samples from adult and pediatric Cystic Fibrosis patients during routine clinic visits and hospitalizations, as well as from normal volunteers with no lung disease
- **Objective 2:** To analyze samples derived from blood, exhaled breath condensate (EBC), nasal curettage, expectorated sputum, and throat swabs using techniques including metabolomics, lipidomics, proteomics, ion chromatography, and measurements of bacterial quorum sensing molecules, markers of oxidative stress, cytokines, and chemokines, and other assays that may be shown to be useful in the future.
- **Objective 3:** To correlate the results from Objective 2 with clinical diagnosis and outcomes in order to determine potential biomarkers that can be used in diagnostic testing that are predictive of the onset of an acute pulmonary exacerbation (APE).

Objective 1

- To generate a bank of well-characterized biological samples from adult and pediatric Cystic Fibrosis patients during routine clinic visits and hospitalizations, as well as from normal volunteers with no lung disease



CF Biorepository

- 447 adult and pediatric CF patients
 - Emory Adult CF Clinic
 - Emory Pediatric CF Clinic
 - Scottish Rite CF Clinic
- 2,558 specimens collected
 - First samples collected on August 26, 2010
- 5,513 aliquots banked



Patient Participants

	Enrolled	Donated	%
Emory Adult	194	156	80%
NDH	114	65	57%
Scottish Rite	138	119	86%
Overall	446	334	75%

Note: Individual clinic numbers do not sum to overall because of patients who have donated at multiple sites

CFBR Database

- **Sample data:**
 - Collection Site
 - Date of Sample Collection
- **Demographic data:**
 - Age
 - Age of CF diagnosis
 - Gender
 - Genotype
 - Genotype class
 - Race/Ethnicity
- **Clinical data:**
 - FEV1 (L and % pred)
 - BMI
 - Current medications
 - Recent microbiology
 - Use of study drugs
 - Secondary complications
 - ABPA, Asthma, Chronic Respiratory Failure, Depression, Hemoptysis, Kidney Disease, Liver Disease, Osteoporosis, Sinus Disease
 - CF manifestations
 - Pancreatic insufficiency, GI disease, Liver disease
 - Number of hospitalizations in total lifetime and over the past 5 years
 - Date of last course of IV antibiotics for an APE
 - Previous surgeries
 - Current symptoms
 - APE at time of collection (Yes/No)
 - Date and results of most recent OGTT
 - CFRD status
 - [hyperlink to encounter in Port-CF](#)



CFBR Database

CFBR : Database (Access 2007 - 2010) - Microsoft Access

File Home Create External Data Database Tools Fields Table

View Paste Copy Format Painter Filter Ascending Descending Remove Sort Selection Advanced Toggle Filter Refresh All Delete More Find Select

Calibri 10 Bold Italic Underline Text Formatting

All Tables Enrolled Patients CRF Samples Patients Approached Normal Consents

Enrolled Pati...	PID	Patient ID#	Age	Collection D	Visit Type	Collection Site	Using Study I	Yes to Inc	Sex	Race-Ethnicity	CFTR
Enrolled Pati...	1	CFBR-105	52	8/26/2010	Initial	EU Hospital	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	1717-10
Enrolled Pati...	2	CFBR-105	52	9/14/2010	Interval	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	1717-10
Enrolled Pati...	3	CFBR-106	22	9/27/2010	Initial	EU Hospital	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Asian or Pacific I	A1319E
CRF	4	CFBR-108	28	9/30/2010	Initial	EU Hospital	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	F508, F5
CRF : Table	5	CFBR-106	22	10/13/2010	Annual	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Asian or Pacific I	A1319E
CRF	6	CFBR-109	37	10/15/2010	Initial	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	177dele
	7	CFBR-107	29	10/19/2010	Initial	Emory Adult CF Clin	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Male	Caucasian	F508, F5
Samples	8	CFBR-099	55	11/2/2010	Initial	Emory Adult CF Clin	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Male	Caucasian	F508, F5
Samples : Tab...	9	CFBR-100	38	11/2/2010	Initial	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	F508, F5
Labeling Sys...	10	CFBR-115	36	11/2/2010	Initial	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	F508, N
Patients App...	11	CFBR-116	35	11/9/2010	Initial	Emory Adult CF Clin	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	F508, G
Patients Appr...	12	CFBR-120	39	11/24/2010	Initial	EU Hospital	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	F508, F5
	13	CFBR-107	29	12/3/2010	SAPE	EU Hospital	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Male	Caucasian	F508, F5
CRF2	14	CFBR-107	29	12/6/2010	SAPE	EU Hospital	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Male	Caucasian	F508, F5
Normal Cons...	15	CFBR-105	52	12/7/2010	Annual	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	1717-10
Normal Cons...	16	CFBR-102	20	1/20/2011	Initial	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Male	Caucasian	F508, R5
NL CRF	17	CFBR-133	44	1/25/2011	Initial	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	F508, Y1
Adverse Eve...	18	CFBR-109	37	1/26/2011	Interval	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	177dele
Adverse Event...	19	CFBR-137	22	1/26/2011	Initial	EU Hospital	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Male	Caucasian	F508, F5
	20	CFBR-110	32	1/28/2011	Initial	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Male	Caucasian	394deIT
	21	CFBR-111	31	1/28/2011	Initial	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	F508, F5
	22	CFBR-121	28	2/2/2011	Initial	EU Hospital	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Male	Caucasian	F508, F5
	23	CFBR-116	35	2/8/2011	Interval	Emory Adult CF Clin	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	F508, G
	24	CFBR-140	41	2/10/2011	Initial	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Male	Caucasian	N/A
	25	CFBR-100	38	2/11/2011	Interval	Emory Adult CF Clin	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Female	Caucasian	F508, F5

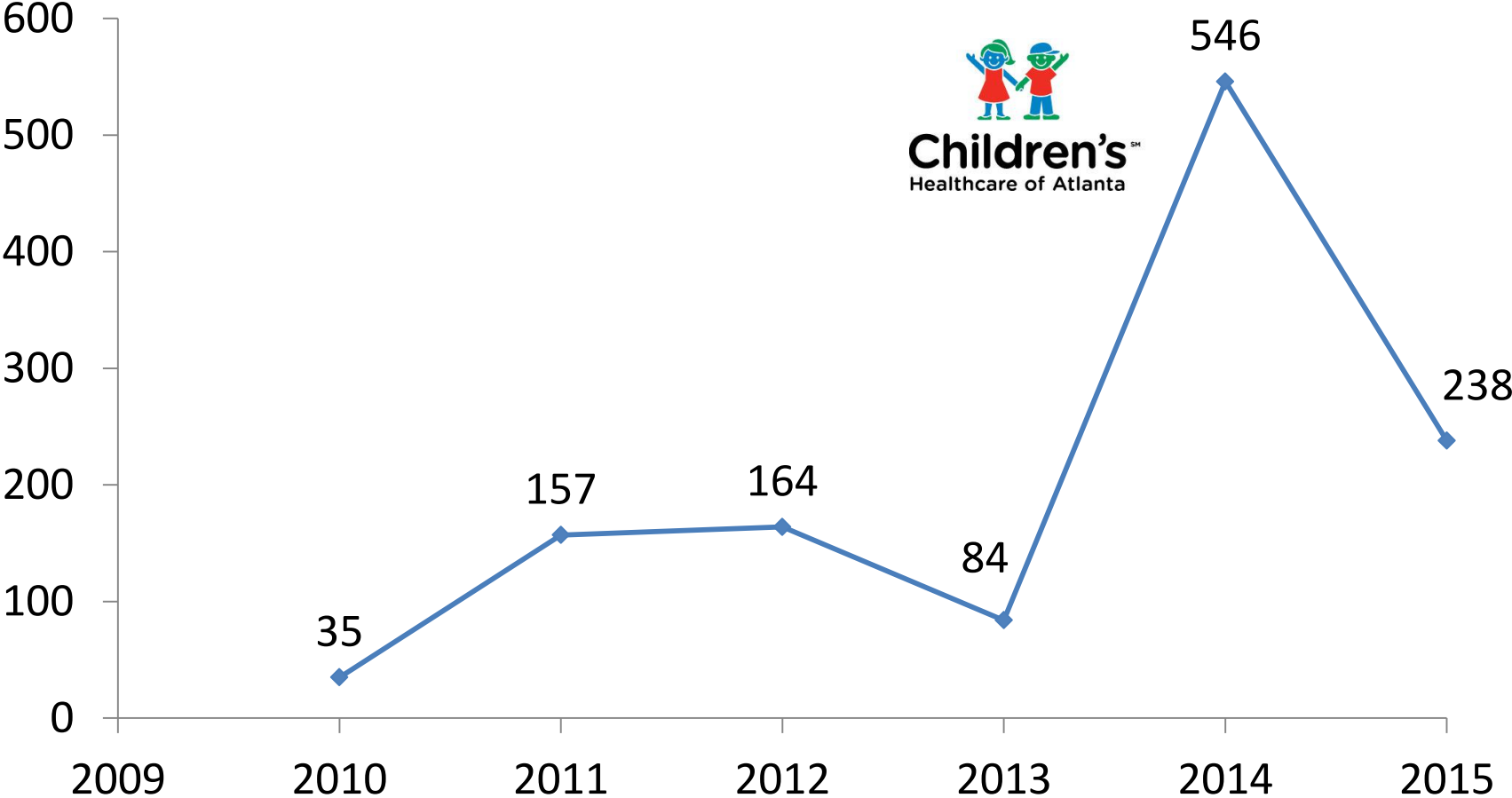
Patient Characteristics

Characteristic	N (%)
Gender	
Female	163 (49)
Male	169 (51)
Genotype (Unknown=14)	
Homozygous F508	159 (48)
Heterozygous F508	120 (36)
Other	41 (13)
Age	
Mean (SD)	19.3 (13.6)
Range	6 months to 70 years
Age of CF diagnosis	
Range	Newborn-63 years
FEV1 % predicted	
Mean (SD)	77.4 (25.0)
Range	18-134
Race	
Caucasian	295 (88)
African-American	14 (4)
Other	11
Pancreatic insufficient	296 (94)
CFRD Status (Missing=100)	
CFRD	65 (19)
Impaired Glucose	26 (8)
Normal Glucose	143 (43)

Specimens in CFBR

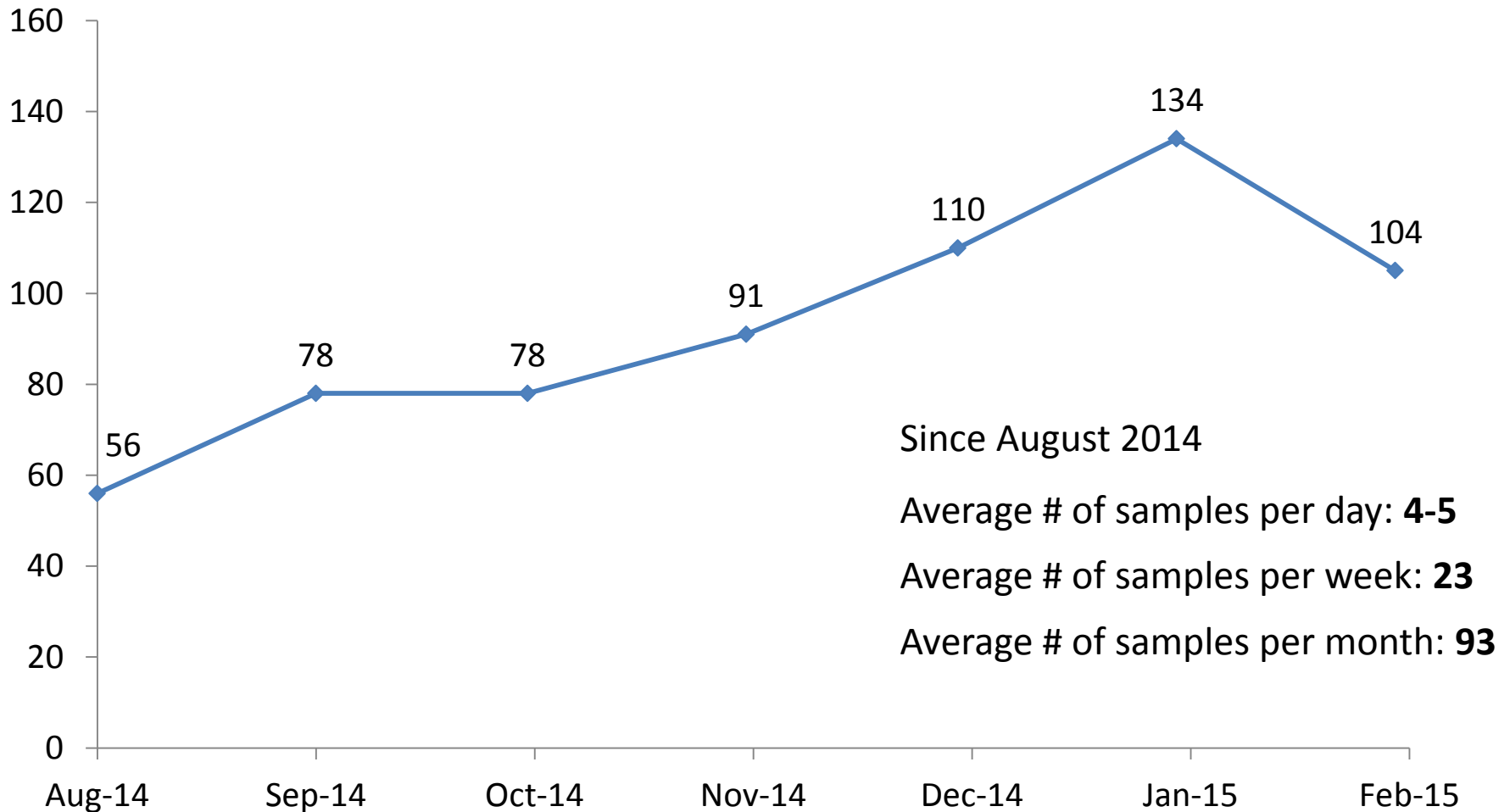
Specimen	Emory Adult	NDH	Scottish Rite	Overall
Bacterial Isolates	1215	122	-	1337
BAL	10	20	7	37
Blood	179	15	63	257
EBC	181	42	82	305
Sputum	398	36	23	457
Throat Swab	1	22	142	165
Total	1984	257	317	2558

Number of Specimens Collected Per Year, 2010-2015



*Excluding bacterial isolates

Number of specimens collected per month, August 2014-February 2015



*Excluding bacterial isolates

Aliquots

Specimen	Overall
Bacterial Isolates	1337
BAL	216
Blood	1134
EBC	813
Sputum	1818
Throat Swab	195
Total	5513

Bacterial Isolates

Bacterial species	N
<i>S. aureus</i>	418
MSSA	42
MRSA	44
Small colony variant	24
<i>Stenotrophomonas maltophilia</i>	68
<i>P. aeruginosa</i>	682
Non-mucoid	14
Mucoid	407
Semi-mucoid	82
<i>Burkholderia cepacia</i> complex	25
<i>Achromobacter xylosoxidans</i>	50
Other	69
Unknown	25
Total	1337

Objective 2

- To analyze samples derived from blood, exhaled breath condensate (EBC), nasal curettage, expectorated sputum, and throat swabs using techniques including metabolomics, lipidomics, proteomics, ion chromatography, and measurements of bacterial quorum sensing molecules, markers of oxidative stress, cytokines, and chemokines, and other assays that may be shown to be useful in the future.



CFBR Utilization

of PI's who have purchased samples: **13**

of aliquots purchased: **639**

Types of projects using CFBR samples:

- Impact of increased RAGE signaling in CFRD Lung Disease
- Investigating the role of lung microbiota during acute exacerbation events, using molecular culture-independent approaches and gene sequencing
- Determine circulating hepcidin-25 levels in CF subjects
- Determine correlation between pyocyanin levels and inflammatory markers in CF sputa
- Rapid detection of *Pseudomonas* pigments in biological fluids using SERS
- CF Microbiome
- Analysis of serum samples of PA-infected CF patients
- Bacterial sphingomyelinase and CFTR
- Neutrophil function in chronic disease
- Proteomics
- AND MORE!!!



Objective 3

- To correlate the results from Objective 2 with clinical diagnosis and outcomes in order to determine potential biomarkers that can be used in diagnostic testing that are predictive of the onset of an acute pulmonary exacerbation (APE).



APE Samples

- 446 (36%) samples in the CFBR currently were collected during an APE
 - Categorize APEs by treatment (APE-O vs. APE-H)
 - Collect information on admission date if patient hospitalized
 - Aim to collect APE-H samples within 48 hours of admission if not before admission while in clinic
- At least 3 longitudinal, prospective studies focused on APEs will be using CFBR samples this year



Longitudinal Sample Collection

	N (%)
# of times patient has donated	
1	119 (36)
2-5	187 (56)
6+	28 (8)
Range	1-23
Follow-up time, years	
Mean (SD)	0.8 (1.2)
Range	0-4.4

Limitations

- Staff
- Processing Space
- Time
- Samples
 - Choose which samples they want to donate and when
 - May not donate the same samples each time
 - Prioritize samples
 - Changes in processing protocols



Summary

- CFBR is a bank of 2,558 well-characterized clinical samples that can be used for CF research studies
- CFBR is rapidly growing
 - Expected to collect 1000+ samples in 2015
- Already being used by the CF research community
- Several studies focusing on APEs will be using CFBR samples this upcoming year
- There are limitations to CFBR
 - May not meet the needs of some studies



Acknowledgments



Principal Investigator

- Nael McCarty, PhD

Co-investigators

- Arlene Stecenko, MD
- Seth Walker, MD, FCCP
- Kevin Kirchner, MD
- Monal Shah, MD
- Rabindra Tirouvanziam, PhD
- Julie Sedor, MD

Scottish Rite Coordinators

- Petra Raville, RN
- Anthony Cantrell

Emory Pediatric Coordinators

- Eric Hunter
- Joy Dangerfield

Other staff

- Derrick Carter
- Chade Granderson
- Carmen Blount, RN
- Barry Imhoff
- Leah Roberts
- Kesmic Jackson, PhD

Previous staff

- Beth Helfman

Clinical care teams at the Emory Adult CF Center, Emory Pediatric CF Center, and Scottish Rite CF Center

All of our patients!!!!!!



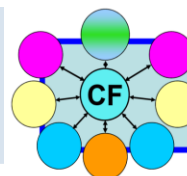
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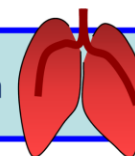


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Advancing Wellness in Patients Through Research





Additional Slides

Year	BAL	Blood	EBC	Sputum	Throat Swab	All samples
2010	0	10	13	12	0	35
2011	0	42	39	76	0	157
2012	10	43	10	101	0	164
2013	16	11	17	40	0	84
2014	9	106	148	158	125	546
2015	2	44	80	73	39	238

Specimen	Frequency	Percent
Sputum	323	34.47
EBC	141	15.05
Throat Swab	105	11.21
Blood	95	10.14
EBC, Sputum	54	5.76
Blood, Sputum	47	5.02
Blood, EBC	41	4.38
Blood, EBC, Sputum	35	3.74
EBC, Throat Swab	30	3.2
BAL	26	2.77
Blood, Throat Swab	23	2.45
BAL, Blood	10	1.07
Blood, EBC, Throat Swab	6	0.64
BAL, Blood, Sputum	1	0.11

Protocols for processing samples

CFBR-_____

Blood :

Processing Start time: _____

Tubes: Red top Lavender Top

0.5 mL into "N" tube for redox; spin for 1 minute & put 200 uL supernatant into "S"

Invert parent tube ~8 times

Spin 1300g for 10 min @ 21°C

Aliquots, serum: _____ Volume: _____

plasma: _____ ; Volume: _____

Stored in Box#: _____ ; -80°C Freezer

Initials: _____

EBC :

240 uL aliquot for redox in "S" tube (with glutathione)

Aliquots: _____ ; Volume in each: _____

Stored in Box#: _____ ; -80 °C Freezer

Initials: _____

Sputum weight= _____ g

Processing Start time: _____

_____ mL D-PBS (w/ EDTA) added

(For every 1 gram of sputum obtained, add 3 mL of EDTA-PBS)

Homogenization: using syringe + 18g needle

(Use 4 cycles of aspiration per gram of initial sputum weight)

Spin 800g for 10 min @ 4°C -Transfer supernatant

Spin 3000g for 20 min @ 4°C

Transfer supernatant; remaining volume= _____ mL

cOmplete Protease Inhibitor Cocktail Solution

_____ tablet(s) in _____ mL D-PBS

_____ mL of remaining volume x 50 µL = _____ µL cOmplete added

Number of Aliquots: _____ Volume of each: _____ mL

Stored in Box# _____; -80°C Freezer

Throat Swab

Processing Start time: _____

Place swab in 15 mL Falcon tube with 2 mL of PBS-EDTA

Leave on ice for 15 minutes

Remove swab

Spin 800g for 10 min at 4°C; Remove supernatant; discard pellet-

Spin supernatant at 3000g for 10 min at 4°C

Remove Supernatant & discard pellet → add Protease inhibitor solution to supernatant

_____ tablet(s) in _____ mL D-PBS

_____ mL of remaining volume x 50 µL = _____ µL cOmplete added

Aliquots: _____ each

Stored in Box # _____; -80°C Freezer