Use of xTAG CF 39 assay for Cystic Fibrosis testing in Ireland

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Dublin, Ireland

xSamples Benelux Symposium
June 19-20

NCMG

Luminex

National Centre for Medical Genetics

- Ireland: population ~4 million
- National Centre for Republic of Ireland
- Three divisions (staff ~70)
  - Molecular Genetics, Cytogenetic, Clinical Genetics
- Molecular Genetics Team (22)
- Molecular tests per year: ~9000 (~50% external)

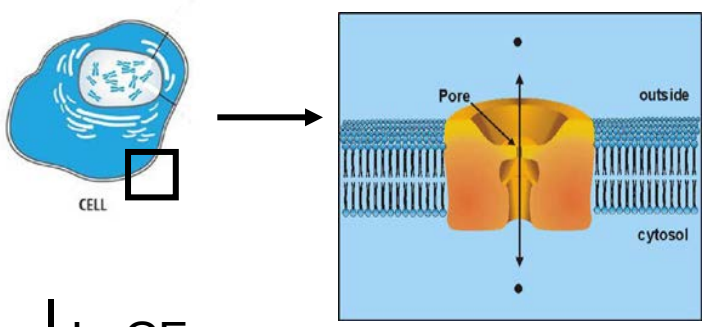
CF in NCMG

- Newborn screening (NBS) for CF in Ireland - 1st July 2011
- CF Numbers increased from
  - ~700 per annum (2010)
  - ~1600 per annum (2012)

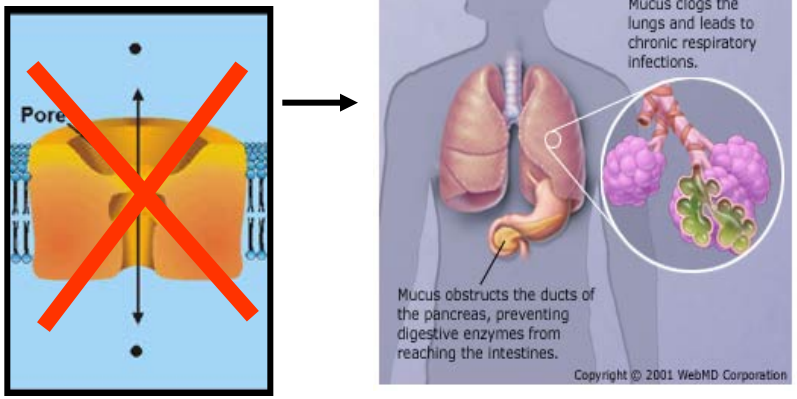
Referral Reason	Sample Numbers 2012
NBS	**825**
Carrier status	**400**
Query affected	**250**
Clinical Diagnosis	**60**
Prenatal Diagnosis	**16**
Predictive test	**10**

- Participation in External Quality Assurance (EQA) schemes for CF and dried blood spot analysis

Cystic Fibrosis – Molecular Pathology



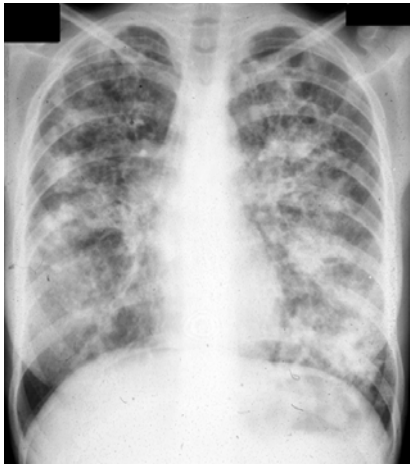
In CF



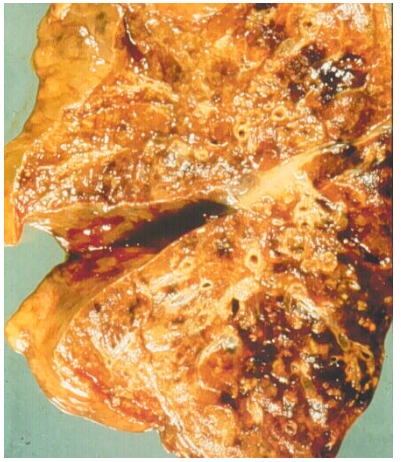
Cystic = Cyst formation
Fibrosis = Thickening & scarring of connective tissue

- CFTR gene, 7q31.2, 27 exons (~230 kb)
- Classical CF
  - Chronic sino-pulmonary disease
  - Gastro-intestinal abnormalities
  - Obstructive azoospermia
  - Pancreatic insufficiency (~85%)
  - Sweat chloride >60mmol/L
  - Two pathogenic mutations
  - Disease progression severe/milder

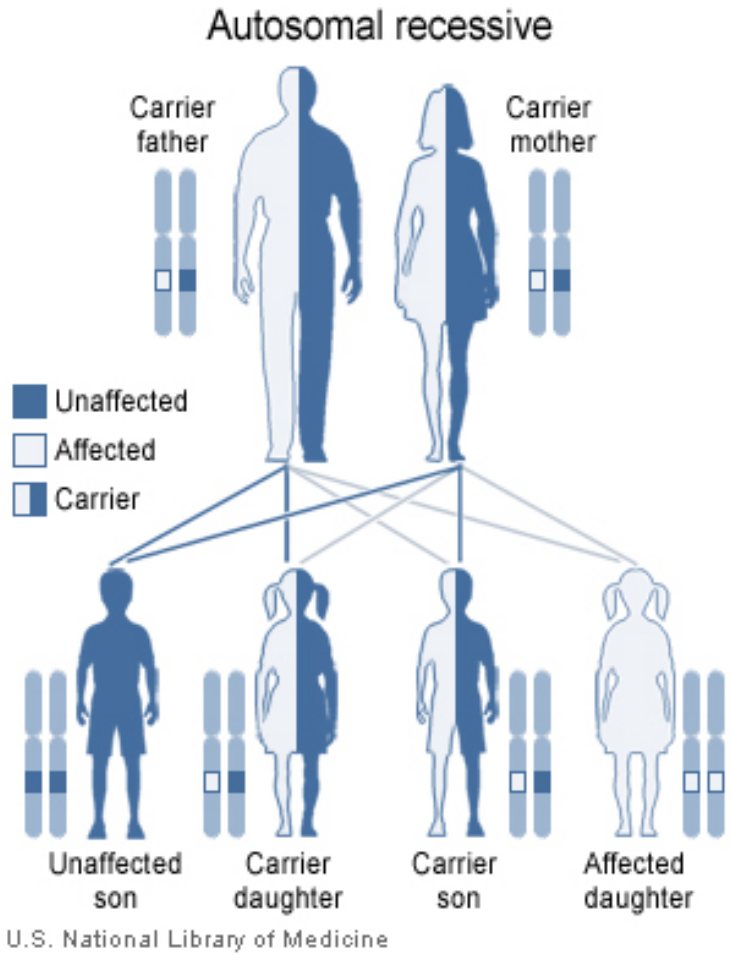
Chest X-Ray



Lung pathology



Cystic fibrosis Genetics



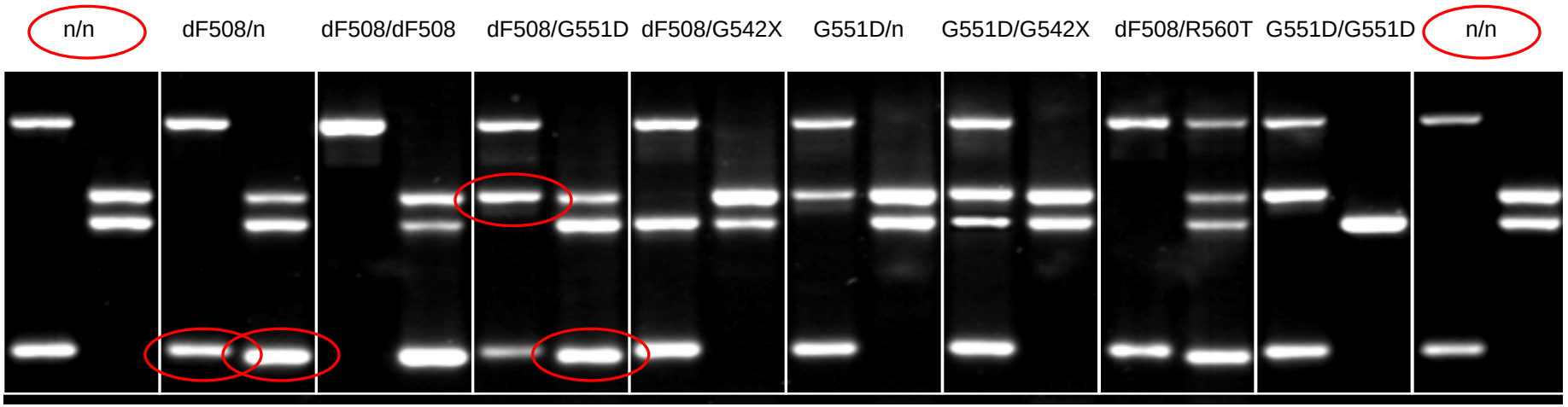
De novo mutations rare in CF

Cystic fibrosis in the Republic of Ireland

- Incidence: 1/1400
- Carrier frequency: 1/19
- 11 of the common mutations account for ~ 92.5%

Mutation	ΔF508	R117H	I507	G542X	G551D	R560T	N1303K	R352Q	1717-1GA	621+1GT	R553X
Frequency (%)	77. 3	2.6	1.0	0.9	6.6	1.9	0.33	0.33	0.33	1.44	0.33

in-House ARMS analysis:



So, WHY change the CF assay?

- ✓ Inexpensive reagents
- ✓ Inexpensive equipment
- ✓ Simple technology & robust (training)
- ✓ Rapid (turn-around time)
- ✓ 93% sensitivity (Irish population)
- ✓ Worked successfully for 15 years

BUT

- Labour-intensive: Several tubes per assay & multiple stages (Poly T analysis)
- Contamination/non-specific bands
- DNA source changing (blood spots)
- Results must be recorded manually
- Changing mutation spectrum (V520F,G85E, 3120+1G>A, 3659delC, 2184delA, W1282X)

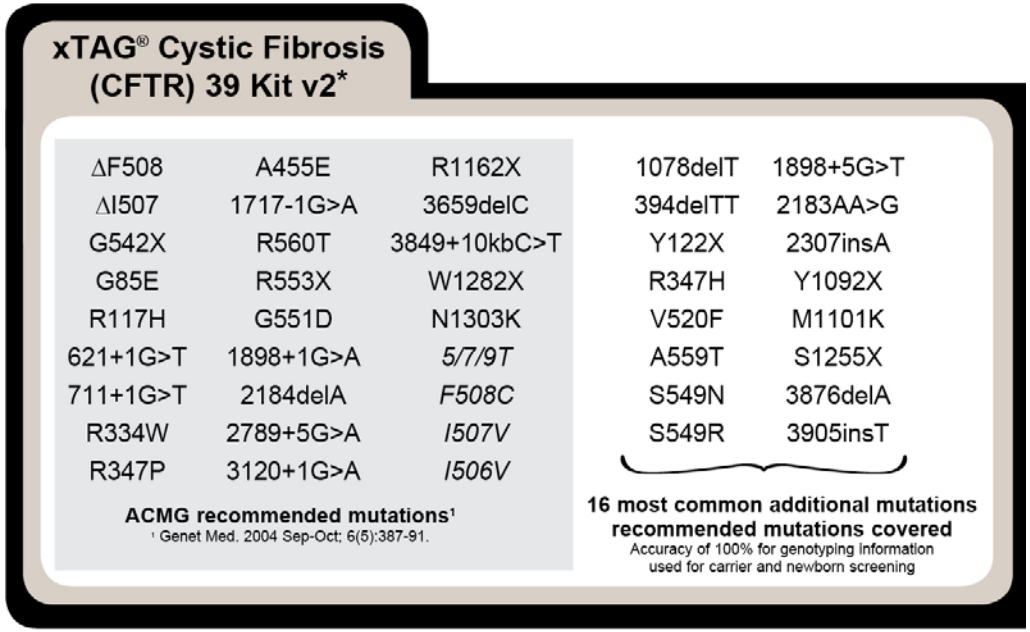
Other kits in use for CF and CF DBS analysis

- CF analysis - UK NEQAS 2011
- CF DBS analysis - UK NEQAS 2012
- CF analysis – CF Network 2012

  - Abbott/Celera OLA (32 mutations)
  - Elucigene CF29 (29 mutations)
  - Elucigene CF-EU2 (50 mutations)
  - Elucigene CF-EU1 (32 mutations)
  - Elucigene CF4 (4 mutations)
  - INNO-LiPA (36 mutations)

Why Luminex / xTAG?

- ✓ Greater mutation coverage than ARMS (ACMG/ACOG 23+)



xTAG® Cystic Fibrosis (CFTR) 39 Kit v2*

ΔF508	A455E	R1162X
ΔI507	1717-1G>A	3659delC
G542X	R560T	3849+10kbC>T
G85E	R553X	W1282X
R117H	G551D	N1303K
621+1G>T	1898+1G>A	*5/7/9T*
711+1G>T	2184delA	*F508C*
R334W	2789+5G>A	*I507V*
R347P	3120+1G>A	*I506V*

ACMG recommended mutations[1]

[1] Genet Med. 2004 Sep-Oct; 6(5):387-91.

1078delT	1898+5G>T
394delTT	2183AA>G
Y122X	2307insA
R347H	Y1092X
V520F	M1101K
A559T	S1255X
S549N	3876delA
S549R	3905insT

16 most common additional mutations recommended mutations covered

Accuracy of 100% for genotyping information used for carrier and newborn screening

List of mutations or variants identified in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene.
**CE-IVD and Licensed for use in Canada.*

Clinical sensitivity of assay increased from 92.5% (ARMS) to 93.5%

- ✓ Single step analysis of classical CF mutations AND 5T/7T/9T & common benign variants
- ✓ Unambiguous homozygous/heterozygous genotype call
- ✓ Single well multiplex assay, minimal transfer steps
- ✓ Integration with patient database
- ✓ High throughput (96-well plate format)
- ✓ Electronic data output & Audit facility
- ✓ Stand-alone instrument – DNA analysers busy!!
- ✓ Technology transferrable
- ✓ Potentially customisable (c.2875delG, NBS - R117H)
- ✓ NCMG Experience with Luminex technology to date – Robust and user-friendly

Information available prior to NCMG validation

- ✓ CE marked, FDA approved
- ✓ Limitations
- ✓ Detailed protocol
- ✓ Optimal DNA concentration (10-1500ng/reaction)
- ✓ Optimal DNA quality (UV 260/280>1.5 (>1.3 for DBS)
- ✓ No interference by Hb, bilirubin, triglycerides
- ✓ Overall accuracy
  - ✓ 98.88-100% (95% CI) for 327 samples
- ✓ Precision & reproducibility
  - ✓ 3 different DNA extraction methods (different sites)
  - ✓ Different operators
  - ✓ Different days

NCMG Validation

- Analytical **Specificity** (100%) – No false positives
- Analytical **Sensitivity** (100%) – No false negatives
  - Specific challenges; G551D/G551D, G551D/R553X, dF508/V520F, dF508/Q493X
- Tolerance of input **DNA range** (10-1500ng/ul)
- **DNA extraction** method & sample type
  - **PureGene** (whole EDTA blood)
  - **EZ1**
    - whole blood
    - blood from neonatal dried blood spots (DBS)
    - CVS tissue
    - cultured/direct amniocytes
  - **phenol/chloroform** (whole blood, CVS tissue, and cultured amniocytes),
  - **whatman** (whole blood)
  - **miniprep** (whole blood)
- **Control DNAs**: Maine Molecular controls (Introl CF Panel II)

MMQCI
MAINE MOLECULAR QUALITY CONTROLS, INC.

10 Southgate Road, Suite 170
Scarborough, Maine 04074 (USA)
Phone: 207-885-1072; Fax: 207-885-1079
www.mmqci.com

TECHNICAL NOTE # 12-002

RE: INTROL™ CF Panel II Control (p/n: G110)
ASSAY: Luminex xTAG™ Cystic Fibrosis 39 Kit v2 (CE-IVD)
DATE: 8/24/12

Below is a table indicating the expected calls for the INTROL™ CF Panel II Control on the Luminex xTAG™ Cystic Fibrosis 39 Kit v2 (CE-IVD).

Variation	Bottle a Call	Bottle b Call	Bottle c Call
G85E	WT	HET	WT
394delTT	WT	WT	HET
R117H	WT	WT	HET
Y122X	HET	WT	WT
621+1G>T	WT	HET	WT
711+1G>T	WT	HET	WT
1078delT	WT	HET	WT
R334W	WT	HET	WT
R347P	WT	HET	Wt D
R347H	WT	Wt D	HET
A455E	WT	HET	WT
dI507	WT or No Call[1]	Wt D	Mu D[2]
dF508	WT or No Call[1]	HET	NS[4]
V520F	WT	HET	WT
1717-1G>A	WT	HET	WT
G542X	WT	HET	WT
S549N	WT	HET or Mu D[2]	WT
S549R(T>G)	HET or Mu D[2]	WT	WT
G551D	WT	HET	WT
R553X	WT	WT	HET
A559T	HET	WT	WT
R560T	WT	HET	WT
1898+1G>A	WT	HET	WT
1898+5G>T	HET	WT	WT
2183AA>G	WT	Wt D	HET
2184delA	WT	HET	Wt D
2307insA	HET	WT	WT
2789+5G>A	WT	HET	WT
3120+1G>A	WT	HET	WT
Y1092X-C>G	Wt D	WT	HET
Y1092X-C>A	HET	WT	Wt D
M1101K	HET	WT	WT
R1162X	WT	HET	WT
3659delC	WT	HET	WT
S1255X(ex.19)	WT	WT	HET
S1255X(ex.20)	WT	WT	HET
3849+10kbC>T	WT	HET	WT
3876delA	WT	HET	WT
3905insT	WT	HET	WT
W1282X	WT	HET	WT
N1303K	WT	HET	WT
5T 7T 9T	7T D or 7T/9T[3] D	7T/9T D	5T / 7T D
I506V I507V F508C	I507V, F508C D	ND	I506V D

- **Repeatability & reproducibility**: Acceptable variation of results between intra runs and inter runs.
- The assay is **robust** as tested by
  - changing operators
  - different break points in process/testing age of PCR products prior to hybridisation
  - use of different thermocyclers
  - using different kit lot numbers
- Delivery of **reagents** is reliable
- Reagents are stable and fit for purpose once opened
- Technical **support** from the Luminex Corp is reliable
- Software design facilitates data checking and **audit**

Summary of Validation

- Consisted of ~500 individual rxn
- 125 DNA samples (normal & mutation positive samples)

DNA – Tissue Types & Concentrations

Sample Type	Conc	Action	Successful on xTAG
EDTA BLOOD • PUREGENE • Qiagen EZ1 • Whatman • Phenol/Chloroform	 100-300ng/ul 50ng/ul 30-50ng/ul 100-300ng/ul	 Diltute to 10ng/ul Dilute 1/5 Dilute to 10ng/ul Dilute to 10ng/ul	 ✓ ✓ ✓ ✓
Dried Blood Spot • Qiagen EZ1	3-5ng/ul	Spin before use. Use Neat.	✓
CVS Tissue • Qiagen EZ1 • Phenol/Chloroform	 20-150ng/ul 50-200ng/ul	 Dilute to 10ng/ul Dilute to 10ng/u	 ✓ ✓

Controls

- Maine Molecular Controls
- Controls for all samples on xTAG CF 39 kit using 3 samples
- Artificial blood samples
- Extract using regular DNA extraction method
- We dilute 1/50 for use

We run these 3 controls on every xTAG analysis

Variation	Call	Raw Signals (MFI)		Background (MFI)		Net Signals (MFI)		Allelic Ratios		
		Wt Allele	Mut Allele	Wt Allele	Mut Allele	Wt Allele	Mut Allele	Wt Allele	Mut Allele	WT
G85E	WT	5799.5	164.0	82.5	74.0	5717.0	90.0	0.98	0.02	0.80
394delTT	HET	5559.0	4737.5	67.5	62.0	5491.5	4675.5	0.54	0.46	0.68
R117H	HET	4356.0	4172.0	59.0	67.0	4297.0	4105.0	0.51	0.49	0.85
Y122X	WT	3779.0	162.0	95.0	70.5	3684.0	91.5	0.98	0.02	0.85
621+1G>T	WT	5104.0	180.5	79.0	48.5	5025.0	132.0	0.97	0.03	0.85
711+1G>T	WT	5290.5	99.0	68.0	70.0	5222.5	29.0	0.99	0.01	0.85
1078delT	WT	6948.5	336.5	72.0	75.5	6876.5	261.0	0.96	0.04	0.80
R334W	WT	5360.5	189.5	80.0	75.0	5280.5	114.5	0.98	0.02	0.75
R347P	Wt D	5183.0	212.0	125.0	83.0	5058.0	129.0	0.54	0.01	0.85
└ R347H	HET		4325.0		75.0		4250.0		0.45	
A455E	WT	5758.0	83.0	52.5	87.0	5705.5	0.0	1.00	0.00	0.85
dI507	Mu D	120.0	2106.0	81.0	72.0	39.0	2034.0	0.02	0.96	0.80
└ dF508	NS		79.0		38.0		41.0		0.02	
V520F	WT	9828.5	231.0	38.0	59.0	9790.5	172.0	0.98	0.02	0.85
1717-1G>A	WT	7147.0	167.5	65.0	73.0	7082.0	94.5	0.99	0.01	0.85
G542X	WT	5727.5	220.0	75.0	53.0	5652.5	167.0	0.97	0.03	0.75
S549N	WT	8602.0	180.0	79.0	72.0	8523.0	108.0	0.99	0.01	0.85
S549R(T>G)	WT	5594.0	151.0	76.0	63.0	5518.0	88.0	0.98	0.02	0.85
G551D	WT	5443.0	94.0	72.0	81.5	5371.0	12.5	1.00	0.00	0.85
R553X	HET	5755.0	3704.0	79.5	75.0	5675.5	3629.0	0.61	0.39	0.85
A559T	WT	6293.5	248.5	74.0	76.0	6219.5	172.5	0.97	0.03	0.80
R560T	WT	8388.0	119.0	67.5	85.5	8320.5	33.5	1.00	0.00	0.85
1898+1G>A	WT	5700.0	131.0	60.0	58.5	5640.0	72.5	0.99	0.01	0.85
1898+5G>T	WT	5559.0	313.0	74.5	73.0	5484.5	240.0	0.96	0.04	0.83
2183AA>G	HET	3342.0	3827.0	89.0	68.0	3253.0	3759.0	0.45	0.52	0.77
└ 2184delA	Wt D		210.0		41.5		168.5		0.02	
2307insA	WT	6901.0	301.0	87.0	63.5	6814.0	237.5	0.97	0.03	0.80
2789+5G>A	WT	4881.5	102.0	57.0	78.0	4824.5	24.0	1.00	0.00	0.85
3120+1G>A	WT	7219.0	149.0	92.0	82.5	7127.0	66.5	0.99	0.01	0.85
Y1092X-C>G	HET	6721.0	6915.0	59.0	75.0	6662.0	6840.0	0.48	0.50	0.75
└ Y1092X-C>A	Wt D		310.5		59.0		251.5		0.02	
M1101K	WT	3932.0	117.0	80.0	63.0	3852.0	54.0	0.99	0.01	0.81

Maximum sensitivity ≥ 96.7% (95% CI)

All Correctly Called

NO FALSE NEGATIVES

90 mutation positive clinical samples

- 5 different extractions methods tested
- 3 plasmid controls, covering 39 mutations

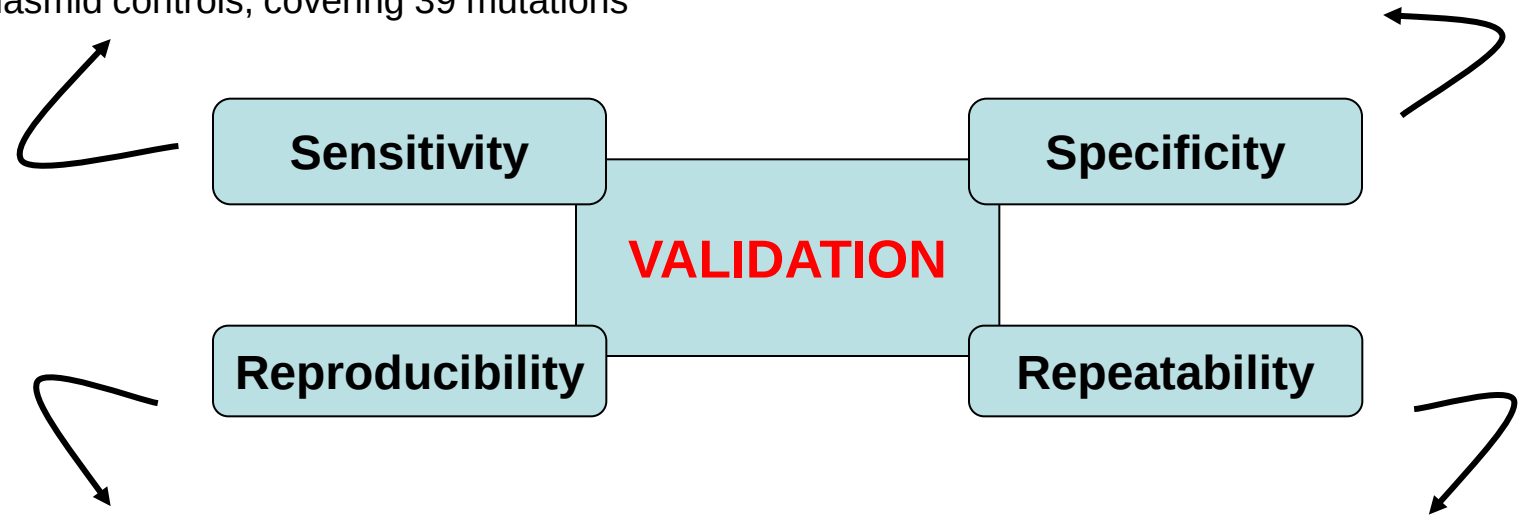
Maximum specificity ≥ 90.0% (95%) (95% CI)

All Correctly Called

NO FALSE POSITIVES

35 WT samples

- 5 different extractions methods tested



9 mutation positive samples

- Run x2 on different plate by a different operator

No inter-run variation in genotype call

9 mutation positive samples

- Run x3 on same plate by same operator

No within-run variation in genotype call

Maternal Cell Contamination

Practice guidelines for the Testing for maternal cell contamination (MCC) in prenatal samples for molecular studies.

'It is ***recommended*** that the chosen MCC assay should routinely be capable of detecting at least a 10% level of MCC'

'Ideally the sensitivity of the MCC assay should be equal to or greater than the specific molecular prenatal assay'

'Where the molecular prenatal test is more sensitive to contamination than the MCC assay it is ***recommended*** that an alternative test …….
….is carried out to confirm the prenatal genotype result'

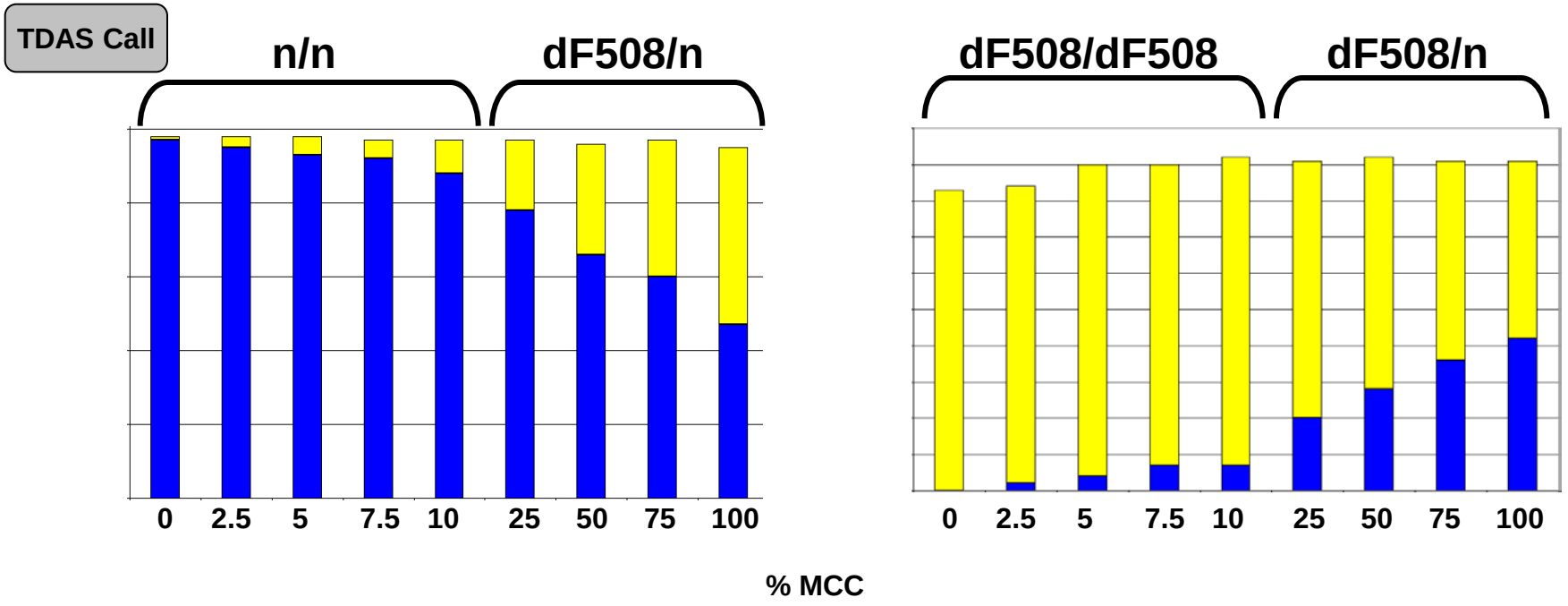
Prenatal Samples & MCC

- Stated that
  - '*The kit is not indicated for use in foetal diagnostic or pre-implantation testing*'
- Need to look closely as Prenatal samples and assess the performance of the kit in the presence of Maternal Cell contamination (MCC)
- 23 sample examined
- No apparent issues with the DNA extracted from Prenatal samples –
  - CVS
  - direct CVS
  - amnio
  - cultured amnio

Extracted by:
Qiagen EZ1
Phenol/Chloroform

ALL GENOTYPES CALLED CORRECTLY

- Spiking Study
  - n/n foetus spiked with 2.5-100% carrier mum
  - Affected foetus spiked with 2.5-100% carrier mum



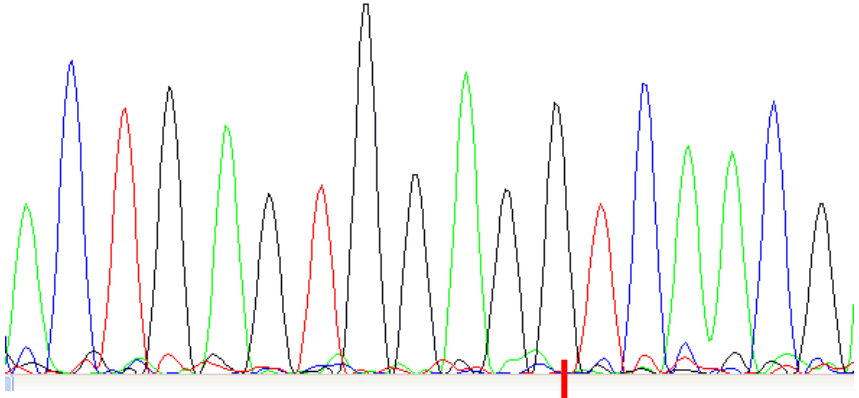
Challenging Genotypes

- Experience indicates potential challenging genotypes for CF assays to deal with, including
  - G551D/G551D
  - G551D/R553X
  - dF508/dI507
  - dF508/V520F
  - dF508/Q493X (Q493X not on xTAG panel, but how would it behave?)

G551D/G551D

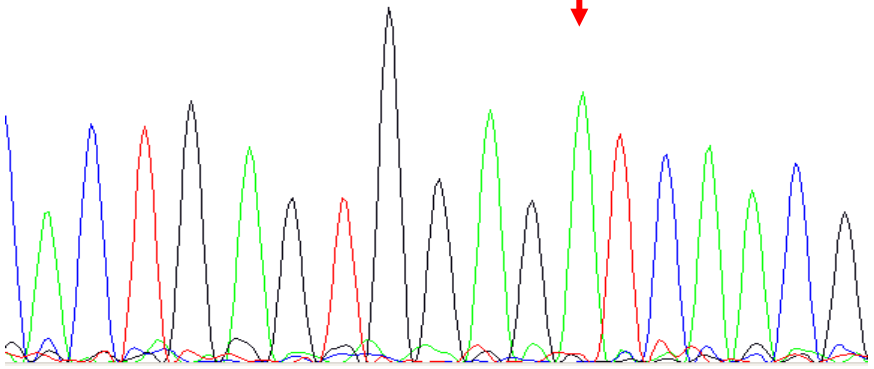
R334W	WT	2823.0	136.0	68.5	31.0	2754.5	105.0	0.96	0.04	0.75	0.28	0.35	
R347P	WT	4962.5	89.0	30.0	25.5	4932.5	63.5	0.99	0.01	0.85	0.27	0.25	
└ R347H	WT		46.0		43.0		3.0		0.00			0.20	
A455E	WT	3433.0	50.0	52.0	46.5	3381.0	3.5	1.00	0.00	0.85	0.25	0.25	
dI507	WT	2248.5	113.0	24.0	30.5	2224.5	82.5	0.96	0.04	0.80	0.20	0.30	
└ dF508	WT		53.5		51.5		2.0		0.00			0.18	
V520F	WT	6074.5	222.5	37.5	33.0	6037.0	189.5	0.97	0.03	0.85	0.25	0.25	
1717-1G>A	WT	2259.5	76.0	48.5	45.0	2211.0	31.0	0.99	0.01	0.85	0.30	0.25	
G542X	WT	3005.0	81.0	31.0	44.0	2974.0	37.0	0.99	0.01	0.75	0.25	0.35	
S549N	WT	1134.0	23.0	52.0	61.0	1082.0	0.0	1.00	0.00	0.85	0.28	0.25	
S549R(T>G)	No Call	109.0	45.5	64.0	33.0	45.0	12.5			0.85	0.25	0.25	Variation failed: signal(s) inadequate
G551D	Mu D	64.0	2874.5	26.0	47.0	38.0	2827.5	0.01	0.99	0.85	0.20	0.25	
R553X	WT	3371.0	20.0	71.0	21.0	3300.0	0.0	1.00	0.00	0.85	0.25	0.25	
A559T	WT	3262.5	50.0	37.0	42.0	3225.5	8.0	1.00	0.00	0.80	0.29	0.30	
R560T	WT	3010.0	77.0	64.0	56.5	2946.0	20.5	0.99	0.01	0.85	0.25	0.25	
1898+1G>A	WT	1925.5	49.0	59.5	54.5	1866.0	0.0	1.00	0.00	0.85	0.25	0.25	
1898+5G>T	WT	2533.5	104.0	75.0	56.0	2458.5	48.0	0.98	0.02	0.83	0.25	0.27	
2183AA>G	WT	2436.0	86.0	56.0	22.5	2380.0	63.5	0.96	0.03	0.77	0.20	0.35	
└ 2184delA	WT		83.5		43.0		40.5		0.02			0.20	

Normal Seq
Forward Direction



Homozygous G>A change

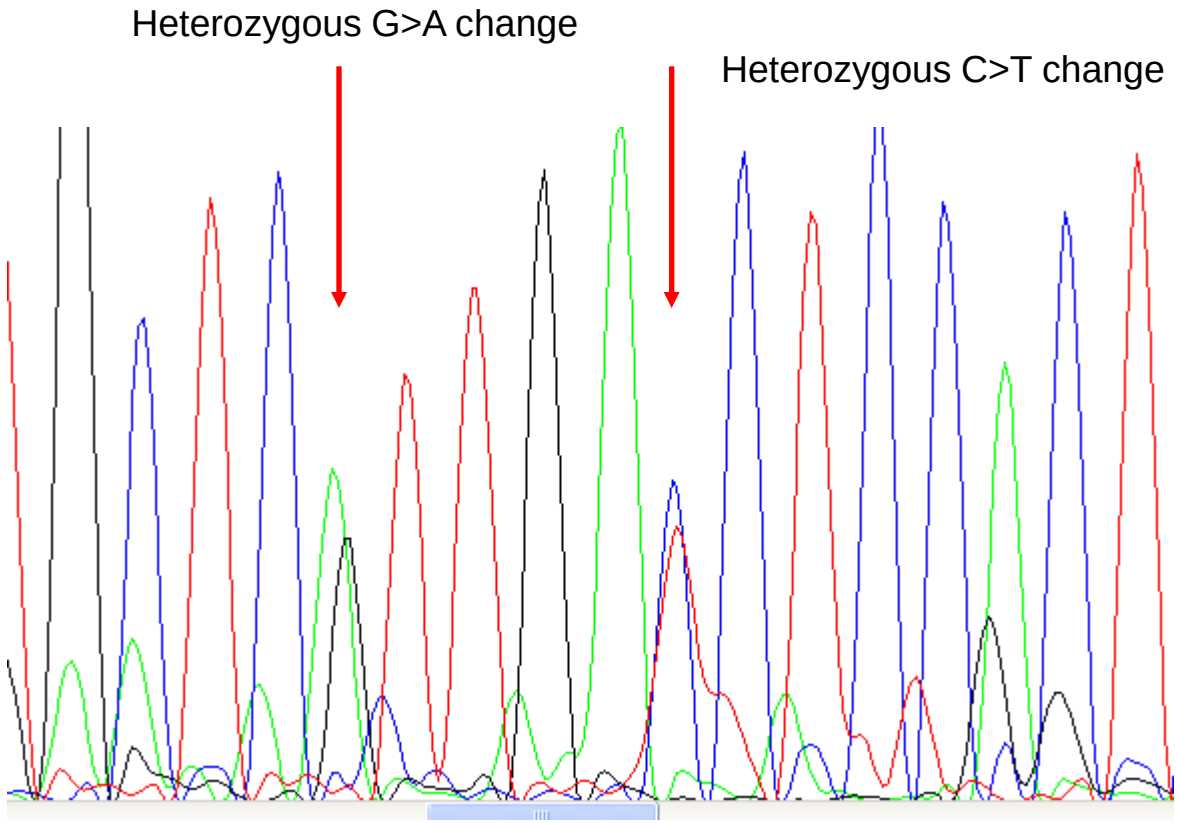
G551D/G551D
Forward Direction



G551D/R553X

dI507	WT	5204.0	157.0	93.0	105.0	5111.0	52.0	0.99	0.01	0.80	0.20	0.30
└dF508	WT		95.0		103.0		0.0		0.00			0.18
V520F	WT	15839.0	297.5	76.0	73.0	15763.0	224.5	0.99	0.01	0.85	0.25	0.25
1717-1G>A	WT	6509.0	188.0	84.0	93.0	6425.0	95.0	0.99	0.01	0.85	0.30	0.25
G542X	WT	7650.0	255.0	76.0	71.0	7574.0	184.0	0.98	0.02	0.75	0.25	0.35
S549N	WT	7508.0	169.5	107.0	70.0	7401.0	99.5	0.99	0.01	0.85	0.28	0.25
S549R(T>G)	WT	2522.0	126.0	73.5	75.0	2448.5	51.0	0.98	0.02	0.85	0.25	0.25
G551D	Mu D	796.5	5106.0	97.0	84.0	699.5	5022.0	0.12	0.88	0.85		0.25
R553X	HET	7520.0	4822.5	92.0	56.5	7428.0	4766.0	0.61	0.39	0.85	0.25	0.25
A559T	WT	7750.5	764.0	70.0	75.0	7680.5	689.0	0.92	0.08	0.80	0.29	0.30
R560T	WT	9829.5	163.0	102.0	90.0	9727.5	73.0	0.99	0.01	0.85	0.25	0.25
1898+1G>A	WT	6142.0	167.0	83.0	102.0	6059.0	65.0	0.99	0.01	0.85	0.25	0.25
1898+5G>T	WT	5738.0	342.0	101.0	67.0	5637.0	275.0	0.95	0.05	0.83	0.25	0.27
2183AA>G	WT	6218.0	245.0	77.0	80.0	6141.0	165.0	0.94	0.03	0.77	0.20	0.35
└2184delA	WT		289.0		84.0		205.0		0.03			0.20
2307insA	WT	8920.0	355.0	90.0	88.0	8830.0	267.0	0.97	0.03	0.80	0.30	0.30
2789+5G>A	WT	6319.0	110.0	74.0	61.0	6245.0	49.0	0.99	0.01	0.85	0.25	0.25
3120+1G>A	WT	7328.5	162.0	88.0	75.5	7240.5	86.5	0.99	0.01	0.85	0.25	0.25

G551D/R553X
Reverse Direction



dF508/dI507

Y122X	WT	3562.0	142.0	69.5	63.0	3492.5	79.0	0.98	0.02	0.85	0.25	0.25
621+1G>T	WT	4687.0	160.0	47.0	47.0	4640.0	113.0	0.98	0.02	0.85	0.25	0.25
711+1G>T	WT	5377.5	121.5	55.5	86.0	5322.0	35.5	0.99	0.01	0.85	0.25	0.25
1078delT	WT	5668.0	354.5	64.0	54.0	5604.0	300.5	0.95	0.05	0.80	0.33	0.30
R334W	WT	3371.5	285.0	66.0	39.0	3305.5	246.0	0.93	0.07	0.75	0.28	0.35
R347P	WT	5725.0	123.0	38.0	38.5	5687.0	84.5	0.98	0.01	0.85	0.27	0.25
└R347H	WT		109.0		65.0		44.0		0.01			0.20
A455E	WT	4423.5	66.0	64.0	45.5	4359.5	20.5	1.00	0.00	0.85	0.25	0.25
dI507	Mu D	63.0	2715.0	49.5	44.5	13.5	2670.5	0.00	0.53	0.80	0.20	0.30
└dF508	Mu D		2457.0		74.0		2383.0		0.47			0.18
V520F	WT	7113.5	254.0	85.0	48.0	7028.5	206.0	0.97	0.03	0.85	0.25	0.25
1717-1G>A	WT	3019.0	150.0	79.0	51.0	2940.0	99.0	0.97	0.03	0.85	0.30	0.25
G542X	WT	3923.0	147.0	75.5	20.0	3847.5	127.0	0.97	0.03	0.75	0.25	0.35
S549N	WT	5946.0	83.0	25.0	65.0	5921.0	18.0	1.00	0.00	0.85	0.28	0.25
S549R(T>G)	WT	3924.5	130.0	50.5	70.0	3874.0	60.0	0.98	0.02	0.85	0.25	0.25
G551D	WT	3691.5	54.0	45.0	43.0	3646.5	11.0	1.00	0.00	0.85	0.20	0.25
R553X	WT	3578.0	72.0	38.0	75.5	3540.0	0.0	1.00	0.00	0.85	0.25	0.25
A559T	WT	3901.0	218.0	41.0	44.5	3860.0	173.5	0.96	0.04	0.80	0.29	0.30
R560T	WT	4393.0	180.0	49.0	36.0	4334.0	84.0	0.99	0.01	0.85	0.25	0.25

dF508/V520F

R334W	WT	3535.0	161.0	47.5	61.5	3487.5	99.5	0.97	0.03	0.75	0.28	0.35
R347P	WT	6111.0	96.0	55.0	51.0	6056.0	45.0	0.98	0.01	0.85	0.27	0.25
└R347H	WT		99.0		49.0		50.0		0.01			0.20
A455E	WT	4220.0	88.0	47.0	43.0	4173.0	45.0	0.99	0.01	0.85	0.25	0.25
dI507	Wt D	2045.0	177.5	34.0	34.0	2011.0	143.5	0.41	0.03	0.80	0.20	0.30
└dF508	HET		2735.0		37.5		2697.5		0.56			0.18
V520F	HET	6081.0	6596.0	75.5	44.0	6005.5	6552.0	0.48	0.52	0.85	0.25	0.25
1717-1G>A	WT	2543.0	71.0	29.0	37.0	2514.0	34.0	0.99	0.01	0.85	0.30	0.25
G542X	WT	3711.5	148.0	40.0	40.0	3671.5	108.0	0.97	0.03	0.75	0.25	0.35
S549N	WT	5079.0	49.0	43.5	39.0	5035.5	10.0	1.00	0.00	0.85	0.28	0.25
S549R(T>G)	WT	3422.0	127.5	42.0	34.5	3380.0	93.0	0.97	0.03	0.85	0.25	0.25
G551D	WT	3302.0	50.5	42.0	43.0	3260.0	7.5	1.00	0.00	0.85	0.20	0.25
R553X	WT	3323.0	90.0	52.0	31.5	3271.0	58.5	0.98	0.02	0.85	0.25	0.25
A559T	WT	3146.0	123.0	43.0	29.5	3103.0	93.5	0.97	0.03	0.80	0.29	0.30

dF508/Q493X

Q493X not on xTAG panel, but does presence interfer with call?

1078delT	WT	5540.5	277.0	69.5	52.0	5471.0	225.0	0.96	0.04	0.80	0.33	0.30
R334W	WT	3065.5	193.0	74.0	86.0	2991.5	107.0	0.97	0.03	0.75	0.28	0.35
R347P	WT	4285.0	105.0	51.0	36.0	4234.0	69.0	0.97	0.02	0.85	0.27	0.25
└R347H	WT		103.5		43.5		60.0		0.01			0.20
A455E	WT	3414.5	81.5	67.0	82.5	3347.5	0.0	1.00	0.00	0.85	0.25	0.25
dI507	Wt D	765.0	98.0	29.5	59.0	735.5	39.0	0.51	0.03	0.80	0.20	0.30
└dF508	HET		695.5		37.5		658.0		0.46			0.18
V520F	WT	4908.0	220.0	42.5	46.0	4865.5	174.0	0.97	0.03	0.85	0.25	0.25
1717-1G>A	WT	2619.5	97.0	58.0	49.5	2561.5	47.5	0.98	0.02	0.85	0.30	0.25
G542X	WT	2803.0	112.0	72.0	68.0	2731.0	44.0	0.98	0.02	0.75	0.25	0.35
S549N	WT	5349.0	64.0	61.0	52.0	5288.0	12.0	1.00	0.00	0.85	0.28	0.25
S549R(T>G)	WT	3217.0	108.0	43.0	59.0	3174.0	49.0	0.98	0.02	0.85	0.25	0.25
G551D	WT	3446.0	50.5	49.0	44.5	3397.0	6.0	1.00	0.00	0.85	0.20	0.25
R553X	WT	3239.0	62.5	58.0	68.0	3181.0	0.0	1.00	0.00	0.85	0.25	0.25
A559T	WT	2698.0	77.5	30.5	73.5	2667.5	4.0	1.00	0.00	0.80	0.29	0.30
R560T	WT	3079.0	80.0	63.0	56.0	3016.0	24.0	0.99	0.01	0.85	0.25	0.25

No, genotype dF508/n, as expected

CF NBS & R117H

- R117H is included on the xTAG CF 39 panel
- Phenotype variable & influenced by 5T/7T background; this cannot be determined without testing the parents to establish phase.
  - Studies have suggested
    - R117H is most commonly found on the 7T haplotype (French Study)
    - Large majority of newborns with R117H and another CF mutation will never develop CF
    - R117H is 10-30 times more frequent in CF 'cases' identified by screening than in those identified by symptoms
    - None of R117H cases identified in a French study* showed any signs of CF by age 7
  - Labels children as having CF who will never develop the disease
  - Lab: further testing e.g. polyT & parental samples
  - Clinical: follow-up

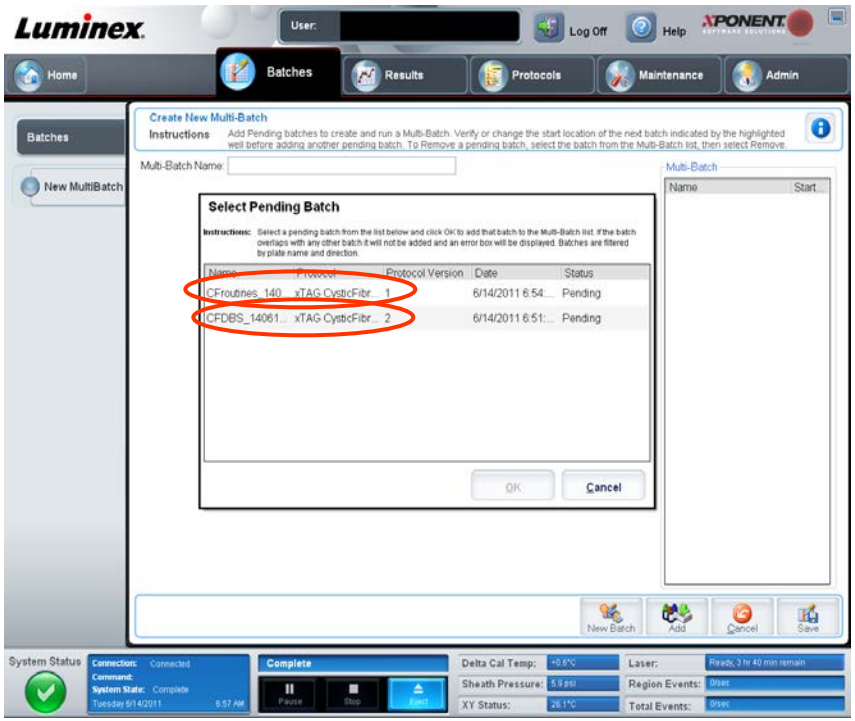
Purpose of NBS is to detect cases of classical CF,
<u>not</u> to pick up cases of atypical CF or CFTR-RD

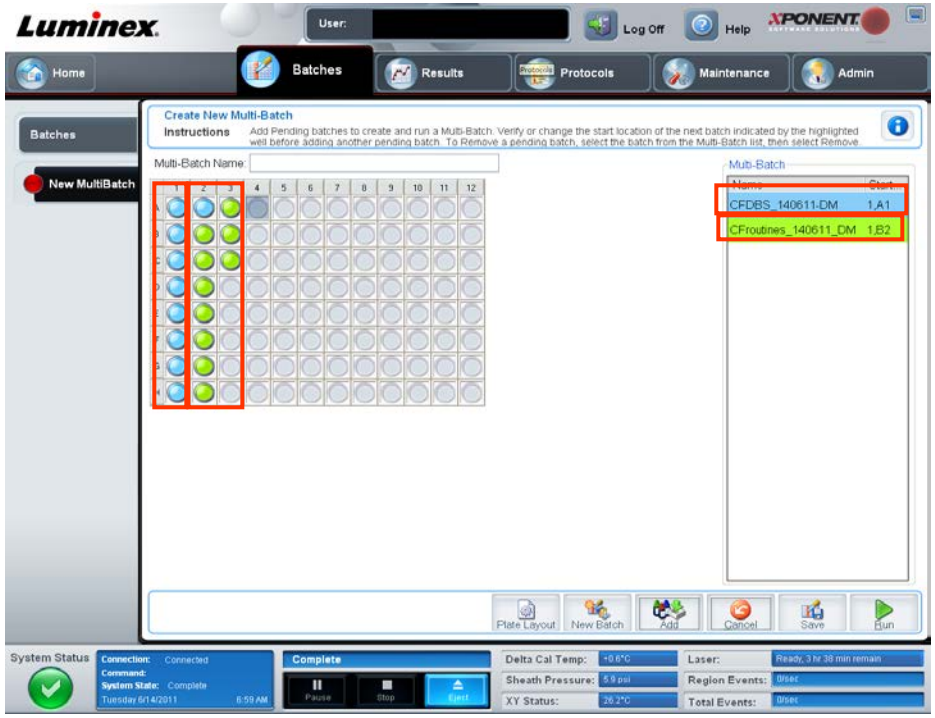
R117H Switched ‘off’

Two Protocol versions implemented

Routine CF samples – Protocol 1
(xTAG 39 – Standard)

NBS samples – Protocol 2
(xTAG CF 39 - R117H ‘Removed’)





Review of Data since 01/01/2013 to 31/05/2013

- 987 samples analysed (including all DBS samples, routine CF & controls)
    - 381 DBS samples
    - 342 Routine CF samples
    - 264 Control samples
- 44 individual set ups
- 4 different operators
  - 1 run repeated
    - *'primary-negative control exceeded acceptable value'*
    - linked to Exo-Sap clean up – likely operator error
  - 2 failed routine EZ1 samples - worked on repeat
    - Very unusual for a EZ1 blood sample to failed
    - Likely insufficient DNA present in well (operator error)
    - Repeat extraction not required
  - 6 failed DBS samples
    - 3 failed as the allelic ratio was outside acceptable range

1717-1G>A	WT	4179.5	143.0	33.0	59.0	4146.5	84.0	0.98	0.02	0.85	0.30	0.25	
G542X	WT	5517.0	121.0	44.0	72.0	5473.0	49.0	0.99	0.01	0.75	0.25	0.35	
S549N	WT	7458.0	149.0	70.5	53.5	7387.5	95.5	0.99	0.01	0.85	0.28	0.25	
S549R(T>G)	WT	4751.0	113.0	56.0	29.0	4695.0	84.0	0.98	0.02	0.85	0.25	0.25	
G551D	WT	5192.0	95.0	80.0	51.0	5112.0	44.0	0.99	0.01	0.85	0.20	0.25	
R553X	WT	5590.5	65.0	62.5	33.0	5528.0	32.0	0.99	0.01	0.85	0.25	0.25	
A559T	No Call	5119.5	1508.0	65.5	63.0	5054.0	1445.0	0.78	0.22	0.80	0.29	0.30	Variation failed: allelic ratio(s) not within predefined ranges
R560T	WT	6139.0	98.0	24.0	34.5	6115.0	63.5	0.99	0.01	0.85	0.25	0.25	
1898+1G>A	WT	4434.5	158.0	58.0	40.0	4376.5	118.0	0.97	0.03	0.85	0.25	0.25	
1898+5G>T	WT	3625.0	172.0	60.5	43.0	3564.5	129.0	0.97	0.03	0.83	0.25	0.27	
2183AA>G	No Call	3966.5	497.5	59.0	31.5	3907.5	466.0	0.76	0.09	0.77	0.20	0.35	Variation failed: allelic ratio(s) not within predefined ranges
└ 2184delA	No Call		855.5		54.0		801.5		0.15			0.20	Variation failed: allelic ratio(s) not within predefined ranges
2307insA	WT	7849.0	462.0	44.5	48.0	7804.5	414.0	0.95	0.05	0.80	0.30	0.30	
2789+5G>A	WT	5007.0	96.0	46.0	65.0	4961.0	31.0	0.99	0.01	0.85	0.25	0.25	
3120+1G>A	WT	4538.5	144.0	45.0	50.0	4493.5	94.0	0.98	0.02	0.85	0.25	0.25	
Y1092X-C>G	WT	6615.0	90.0	45.0	39.0	6570.0	51.0	0.97	0.01	0.75	0.25	0.30	
└ Y1092X-C>A	WT		227.0		73.0		154.0		0.02			0.30	
M1101K	WT	3929.5	181.0	55.5	47.0	3874.0	134.0	0.97	0.03	0.81	0.25	0.29	
R1162X	WT	5285.0	122.0	62.5	39.0	5222.5	83.0	0.98	0.02	0.85	0.30	0.25	
3659delC	WT	5446.5	231.0	43.0	79.0	5403.5	152.0	0.97	0.03	0.81	0.31	0.29	

- Remaining 3 failed due to inadequate MFI signal, either on all beads or individual beads
- All worked well on repeat, no re-extraction required

8/987 = 0.8% fail rate

2/342 EZ1 samples; 0.5% fail rate

6/381 DBS samples; 1.5% fail rate

Summary of Validation

- ✓ Validation successful
- ✓ Using xTAG CF 39 kit on live samples since April 2011
- ✓ Works really well on dried blood spots for NBS
- ✓ All validation parameters met
- ✓ Highly Recommended!

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