A pilot study for the application of Next Generation Sequencing in Cystic Fibrosis Newborn Screening

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Current protocol – 'IRT-DNA-DNA-IRT'

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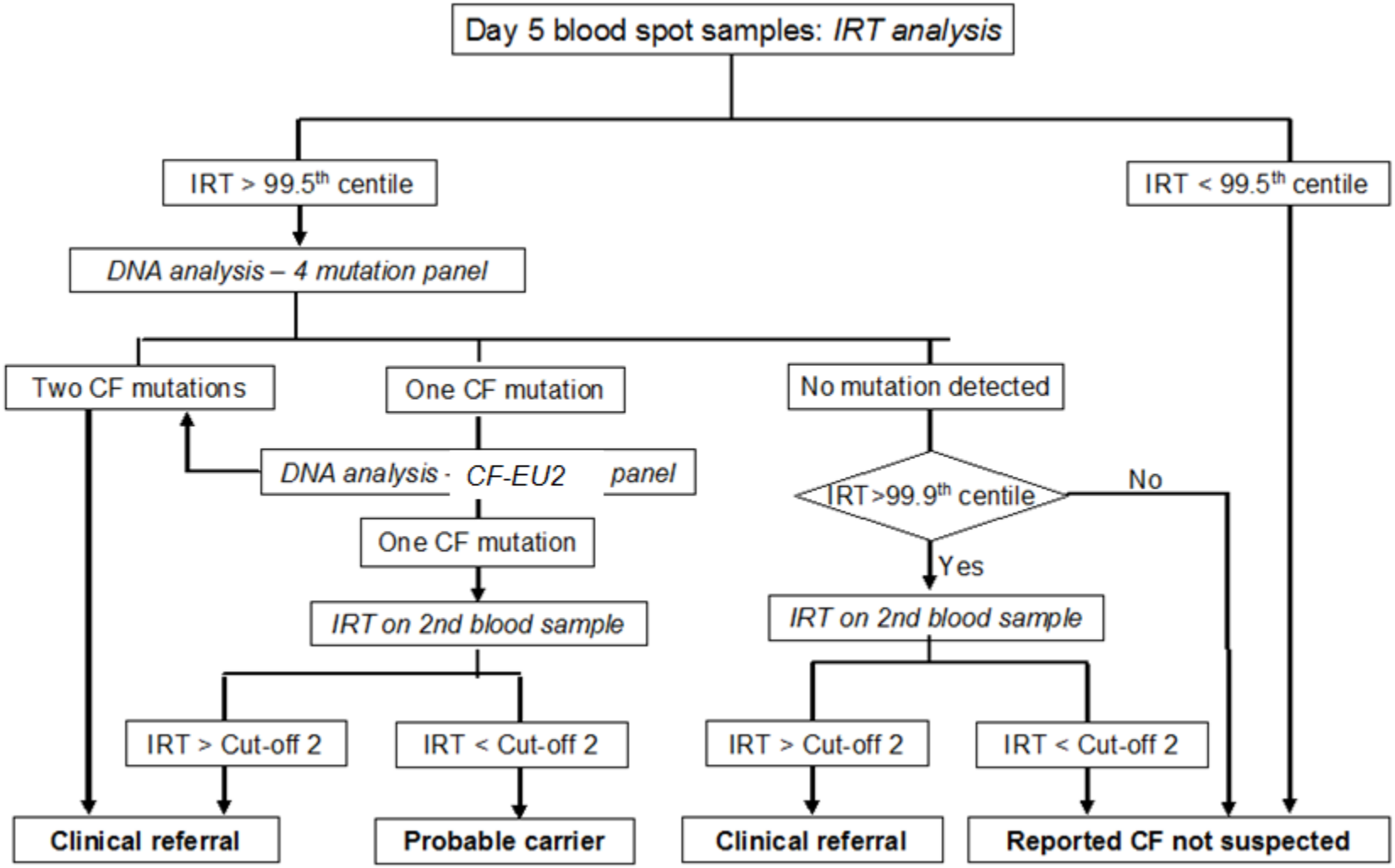

Potential of NGS for CF NBS

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- Pros of our current approach
  - Protocol/programme currently works well
  - 97% sensitivity
- Cons of our current approach
  - 77 carriers identified for every 100 confirmed cases
  - 91% babies requiring 2nd bloodspot turn out to be CF not suspected
- Why introduce NGS?
  - Increased sensitivity, particularly for babies of 'non-caucasian' ancestry as the range of pathogenic variants covered can be increased without significant increase in cost.
  - Reduce number of babies requiring 2nd heelprick/bloodspot
  - Ultimately, eliminate identification of carriers

→ **1 year pilot study, 75,000 babies began August 2018**

Pilot study

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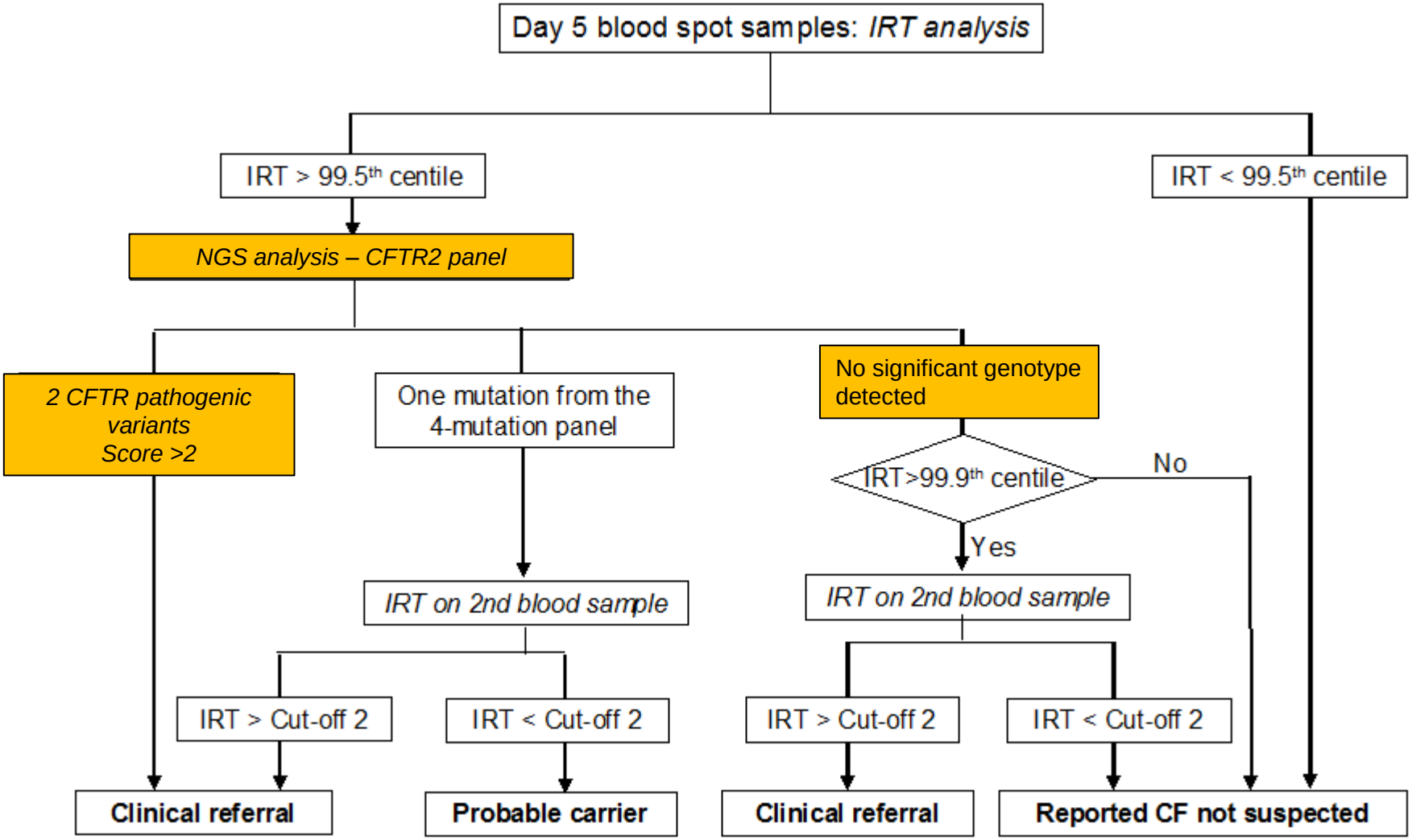

Weekly workflow

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Bloodspots/DNA
- Genomic DNA extracted from bloodspots (EZ1 – Qiagen)

THURS

NGS of CFTR gene
- AmpliSeq community CFTR panel (Thermo Fisher)
- Ion S5 XL (Thermo Fisher)

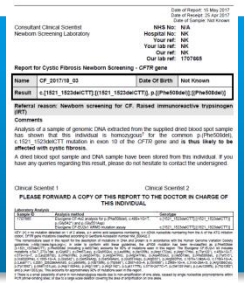
FRI/ SAT

MON

Analysis Pipeline
- Bespoke bioinformatics pipeline
- 332 pathogenic variants (CFTR2)
- 'Significant genotype' scoring algorithm

MON/ TUES

Review/reporting
- Web-based interface
- (Confirm pathogenic variants)
- Issue reports



Genotype scoring algorithm

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Variant description	Examples	Score
CF-causing	p.Phe508del, p.Gly542*, p.Arg117His-5T	2
Varying clinical consequence	p.Asp1152His, p.Arg117His-7T	1
Non-CF causing/ unknown significance	p.Arg31Cys, p.Ile148Thr	0 (excluded from panel)

'Significant genotype' - Only samples with a total/genotype score >2 are reported (*except* for carriers of one of the 4 common pathogenic variants).

Bioinformatics analysis pipeline

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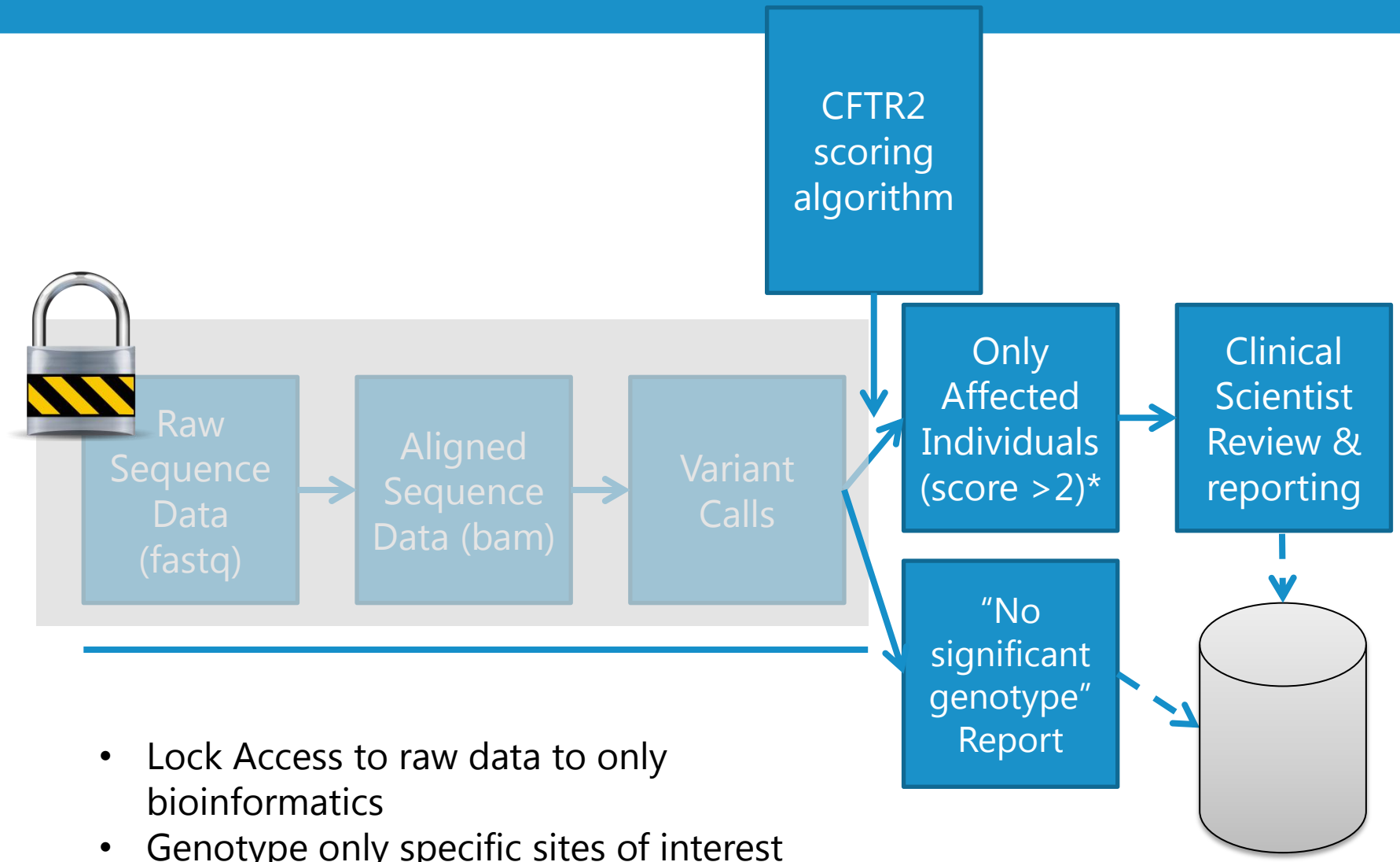

- Lock Access to raw data to only bioinformatics
- Genotype only specific sites of interest

* Plus carriers for one of the 4 common pathogenic variants

CF Status Check

Worklist 1809761

10 CF patients on worklist 1809761

☑ AFFECTED ☑ CARRIER_REPORT ☑ NO_SIG_GENOTYPE ☑ NOT TESTED

Sample	CF Status	Gaps File	Path to Results
S1826345-02	AFFECTED	PASS	P:\S1826345-02 Copy path
S1826346-02	AFFECTED	PASS	P:\S1826346-02 Copy path
S1826351-02	CARRIER_REPORT	PASS	P:\S1826351-02 Copy path
S1826347-02	NO_SIG_GENOTYPE	PASS	
S1826348-02	NO_SIG_GENOTYPE	PASS	
S1826349-02	NO_SIG_GENOTYPE	PASS	
S1826352-02	NO_SIG_GENOTYPE	PASS	
S1826354-02	NO_SIG_GENOTYPE	PASS	
S1826356-02	NO_SIG_GENOTYPE	PASS	
S1826357-02	NO_SIG_GENOTYPE	PASS	

Download Results

Data from Pilot study 1/8/18 to 30/4/19

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- 54,000 samples analysed
- 215 samples with IRT >99.5th sent for DNA analysis
  - 14 samples 'failed' on NGS (6.5%), so reported using CF4/EU2 only
  - 37 non-normal CF outcomes in this time
    - >>>

Non-normal samples (n=37)

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- 12 probable carriers (i.e. one pathogenic variant from CF4 + low second IRT)

- 25 referrals to paediatric specialist team "CF suspected"

  16 would have been detected by the existing approach
  - 13 with 2 pathogenic variants on CF4/EU2 panel
  - 1 with 1 pathogenic variant from CF4 + high second IRT
  - 2 with no significant genotype but high second IRT (initial >action limit 2)

  9 were detected by use of NGS >>>

9 referrals due to NGS use

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	Variant 1	Varant 2	Variant locations	Comments
1	ΔF508	c.297-3C>T; 4279insA	1 CF4, 1 NGS	Diagnosed antenatally (sibling affected). Sweat chloride 89.
2	1154insTC	5T (11TG)	2 NGS	CFSPID. Sweat chloride 21.
3	R117H (7T)	3849+10kbC>T	2 CF-EU2	CFSPID. Sweat chloride 27.
4	c.1029del	5T (11TG)	2 NGS	CFSPID. Sweat chloride 43.
6	ΔF508	5T (12TG)	1 CF4, 1 NGS	Complex referral - no outcome data
7	ΔF508	5T (11TG)	1 CF4, 1 NGS	Feedback awaited. Repeat IRT not requested.
5	ΔF508	F1052V	1 CF4, 1 NGS	Repeat DBS requested. Sweat chloride 10.
8	R117H (7T)	4326delTC	1 CF-EU2, 1NGS	Feedback awaited
9	ΔF508	R1070W	1 CF4, 1 NGS	Repeat IRT 91 (52). 2 previous sweats insufficient.

2nd IRT referrals – data from elsewhere

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Data from other labs – a retrospective look at positive cases from 2nd bloodspot/IRT referrals

- Lab 1 (data from 2006-2018):
  - 35 with no or 1 mutation and CF confirmed – 22 would have been picked up using NGS (63%)
- Lab 2 (data from 2008-2018):
  - 23 with no or 1 mutation and CF confirmed – 18 would have been picked up using NGS (78%)

Conclusions?

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- Technically feasible but challenging
  - Turnaround times OK
  - Failure rate reducing
  - Cost acceptable
- Avoids the need for a second IRT in 60-70% of cases, however some cases would be missed with the current NGS panel without 2nd IRT
- Some cases referred have an uncertain significance (CFSPID) – could be avoided by not reporting variants of varying clinical consequence
- Potential to adapt the panel and modify how it is used
- Insufficient evidence of benefit to adopt as it stands…