The Development, Testing and Rollout of Lateral Flow Testing in the UK

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Reasons to Test for Covid

- Is covid the cause of current illness?
- Has individual recently had covid?
- Is individual immune or susceptible to covid?
- **Is individual currently infectious?**

Control Methods against Covid19 Epidemiological Tools

Universal Precautions

- Decrease rate of transmission from all infectious individuals (background)
  - Universal social distancing
  - Use Face marks / hand washing
  - Avoidance of skin-to-skin contact (outside households)

Quarantine of individuals at 'high risk' of being infectious

('guilty by association' – unpopular and expensive) **quarantine-days/tx**

- Lock down of regions with high disease incidence 1000
- Quarantine of contacts of known positives 70

- Individual assessments
  - Quarantine of individuals with symptoms suggestive of covid19 70

Methods to identify Infectious Individuals
Use of PCR tests

- Widespread confidence in PCR and is considered to be synonymous with the disease!
- Practical Difficulties
  - Requires laboratory and trained staff
  - Expensive (Mass testing facilities have now been stood down)

Problems of Interpreting PCR test

- Recent NP swab with positive PCR result (categorical pos/neg result)
  - High False Positive Rate for infectiousness (c30-50%) in asymptomatics
    - PCR detects RNA fragments as well as intact virions (clearance of viral fragments takes weeks)
    - Risk of laboratory contamination leading to clusters of false positives
  - False negative rate during periods of high transmission
    - Long turn round time rates mean that individuals can become infectious during the 1-2 days that they wait for results.

Control Methods against Covid19
Epidemiological Tools + PCR

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  - Quarantine of known PCR+ individuals (regardless of viral load) 20
  - Quarantine PCR+ at the time of onset of covid symptom 5

Quarantine/Lock Down Policy Sensitivity

Misses infectious individuals

- About 30-50% of covid infections are entirely asymptomatic and many are asymptomatic in the early stages of the infectious period
- Need for test for ‘non-infectious’ individuals to allow release from quarantine/lockdown

Are Lateral Flow Tests better at detecting infectious individuals?

Lateral Flow Device (LFD) Antigen Tests

- ‘Pregnancy Test’ type approach.
- Essentially suitable for home use
- Measures viral protein with monoclonal antibodies
- In principle the technology gives highly specific and sensitive results
- Results in within 30min (depending on manufacturer)

Relationship between viral load and infectiousness

- Culturing virus is difficult. Infectious dose of virions is not clear.
- Are different body compartments differentially infectious (saliva, nose, pharynx, lungs, stool)?
- How do viral swab viral load relate to aerosol/droplet spread?

The Challenge of Evaluation of LFD

- No standard covid antigen calibrant available to assess LFD devices.
- qPCR detects RNA and is used as a surrogate for antigen
  - PCR is not licensed for quantitative load.
  - Each laboratory have different CT values – sometimes very different.
  - Conversion to viral loads is difficult
- Underlying assumption that there is a simple relationship between concentrations of RNA and of antigen.
  - Live infectious viruses
    - Culturable virus
  - Dead virus (RNA+protein shell)
  - RNA only (RNA fragments)
  - Empty shells (Antigen only)

UK Evaluation of Lateral Flow Antigen Devices

- Oxford – PHE Porton collaboration was set up to evaluate different Lateral Flow kits.
  - DHSC sent kits that passed initial ‘due diligence’ screening
    c150 kits were sent to Porton
- Stage 1: Initial Screening
  - Test against standard cultured virus (plaque forming units/ml) and negative tests.
  - Test against common cold coronavirus

Table 1

Limit of detection for SARS-CoV-2 detection by the Innova LFD for antigen detection using saliva sample spiked with SARS-CoV-2. Ct - cycle threshold. PFU - plaque forming units.

PFU/ml	Ct equivalent	Positive LFD tests/total LFD tests	% positive
100,000	16	20/20	100
10,000	19	25/25	100
1000	23.7	65/65	100
390	25.2	5/5	100
100	25.5	63/65	96
40	28.5	3/5	60
20	29.3	0/5	0
10	30.2	0/5	0
5	31	0/5	0
2.5	31.7	0/5	0
1.2	32.5	0/5	0

Peto et al EClinical Medicine (2021) 36:100924

UK Evaluation of Lateral Flow Antigen Devices Stage 2

- Bank of 200 samples from positive individuals with a range of qPCR-defined viral loads were stored (carefully calibrated)
- 1000 negative samples for specificity
- Allows direct comparison with other kits.
- All kits evaluated for kit failures and ease of use.
- Methodological note
  - Swabs were placed in 3ml fluid for PCR and the same fluid was used for LFD assessment
  - *LFD field testing puts swab in c200ul of fluid to maximize sensitivity*

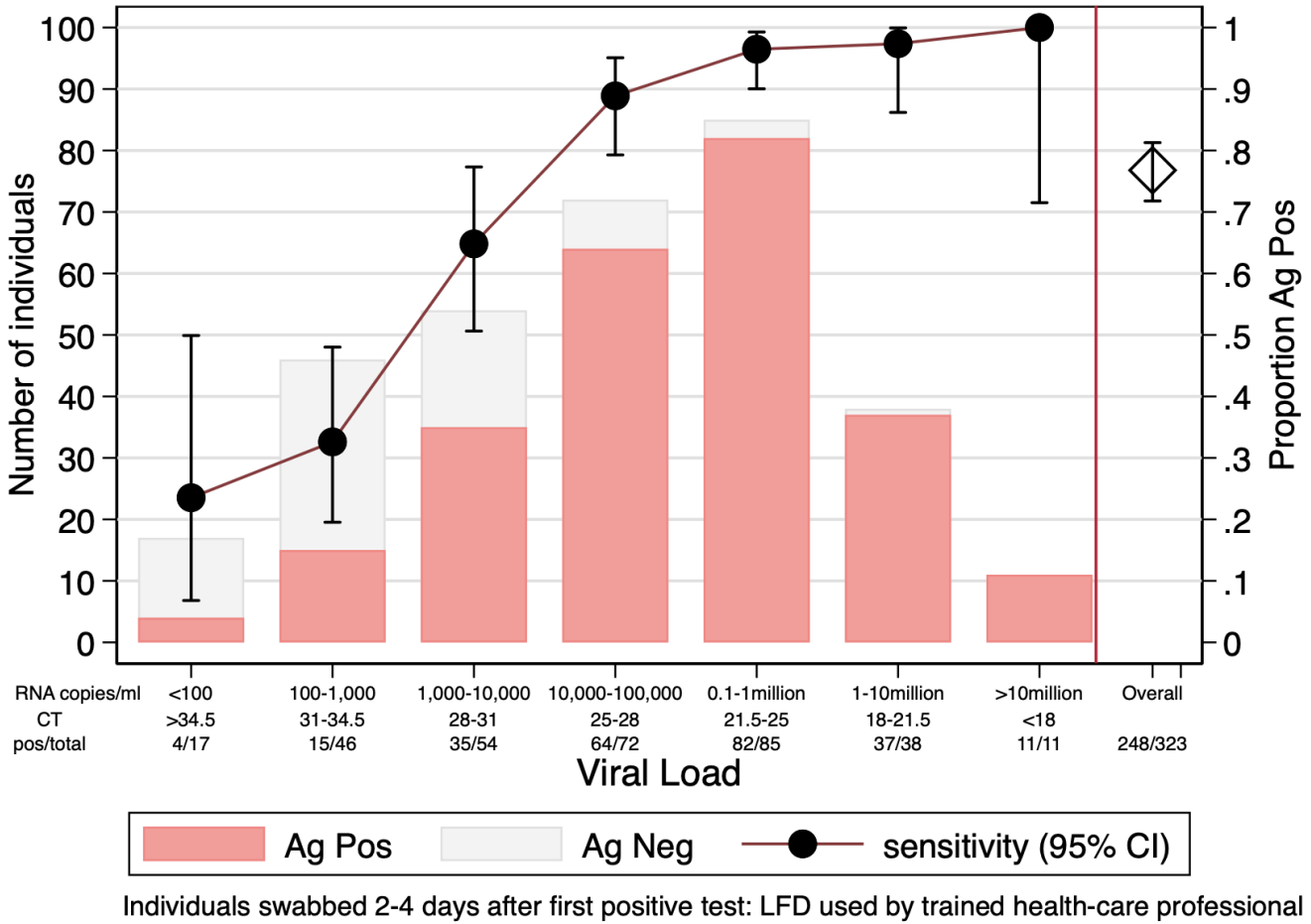
Results form 200 stored samples comparing different kits

Viral load	ORF1 CT	Innova	Abbott	Orient gene	Deepblue	Fortress	SD Bio swab	Surescreen
>10million	<18	3/3 (100.0)	3/3 (100.0)	3/3 (100.0)	3/3 (100.0)	3/3 (100.0)	3/3 (100.0)	3/3 (100.0)
1-10 million	18-21.5	25/25 (100.0)	28/28 (100.0)	27/27 (100.0)	28/28 (100.0)	28/28 (100.0)	27/27 (100.0)	28/28 (100.0)
0.1-1 million	21.5-25	31/33 (93.9)	33/35 (94.3)	32/35 (91.4)	32/35 (91.4)	31/35 (88.6)	33/34 (97.1)	33/34 (97.1)
10,000-100,000	25-28	23/34 (67.6)	25/37 (67.6)	26/37 (70.3)	23/37 (62.2)	28/37 (75.7)	19/36 (52.7)	16/37 (43.2)
1,000-10,000	28-31	12/41 (29.3)	13/42 (31.0)	5/42 (11.9)	4/42 (9.5)	15/42 (35.7)	7/42 (16.7)	0/42 (0.0)
100-1,000	31-34.5	1/37 (2.7)	1/41 (2.4)	0/41 (0.0)	0/41 (0.0)	2/41 (4.9)	0/41 (0.0)	1/40 (2.5)
<100	>34.5	0/5 (0.0)	0/5 (0.0)	0/5 (0.0)	0/5 (0.0)	0/5 (0.0)	0/5 (0.0)	0/5 (0.0)
Negative samples	na	0/940 (0.0)	5/1589 (0.003)	0/999 (0.0)	0/1014 (0.0)	1/1000 (0.001)	1/996 (0.001)	1/995 (0.001)

UK Evaluation of Lateral Flow Antigen Devices Stage 3

- Field Testing in Test and Trace.
  - Some Individuals stated that they were asymptomatic.
- Two swabs were taken.
  - One swab was placed in 3ml of viral transport medium and tested with conventional PCR.
  - One swab was placed in 6 drops (c200ul) of fluid and assessed by LFD.
- Variation between swabs not considered.
- Different techniques of swabbing not clear. Studies are inconsistent.

Experienced Users: Proportion Individuals Ag Positive by their Viral Load



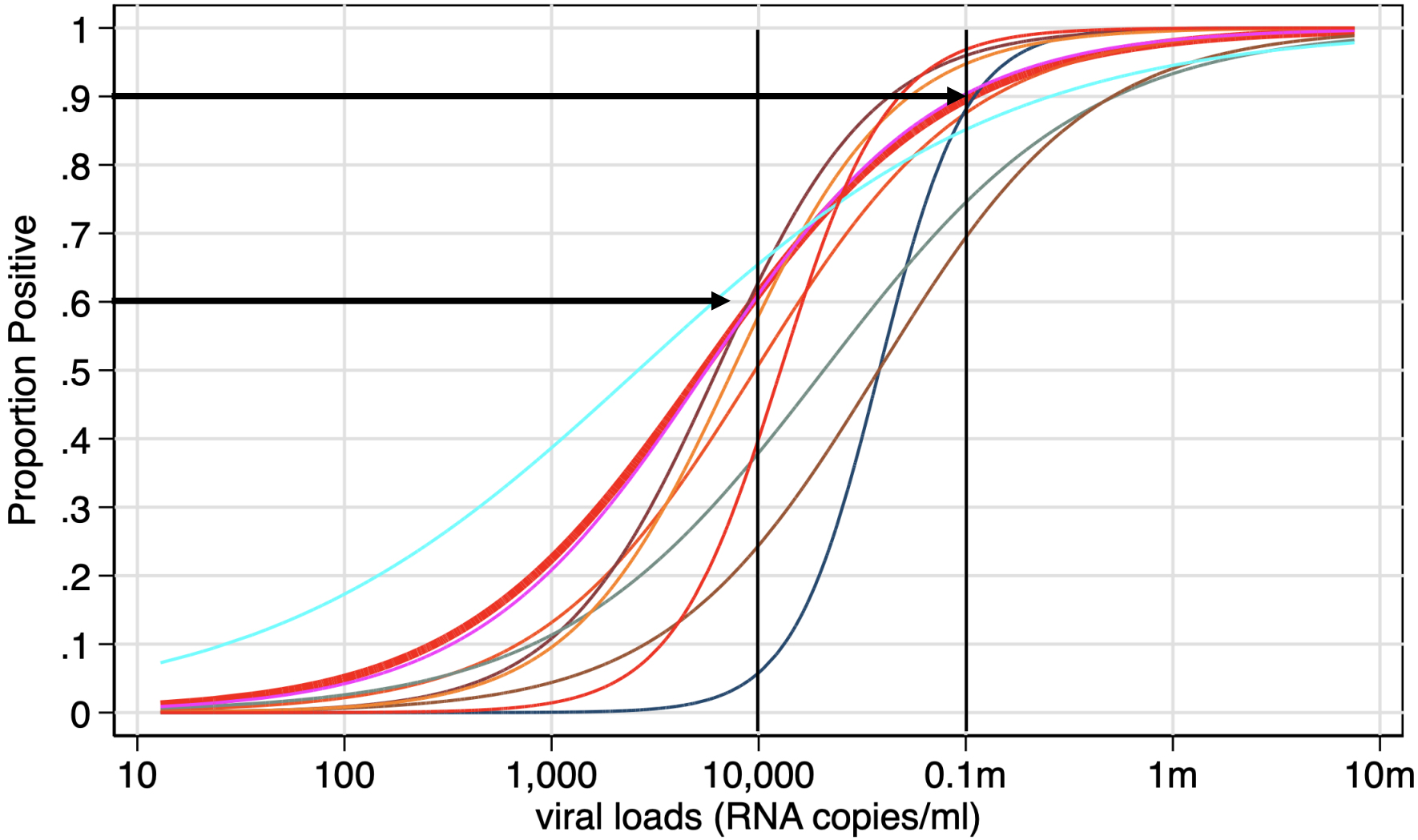
Individuals swabbed 2-4 days after first positive test: LFD used by trained health-care professional

Peto et al EClinical Medicine (2021) 36:100924

How to compare performance of different Lateral Flow Kits

- No single figure can easily summarize the relationship between lateral flow result and viral load.
- Logistic regression model is used to describe the relationship

Comparison of 9 LFDs with Innova

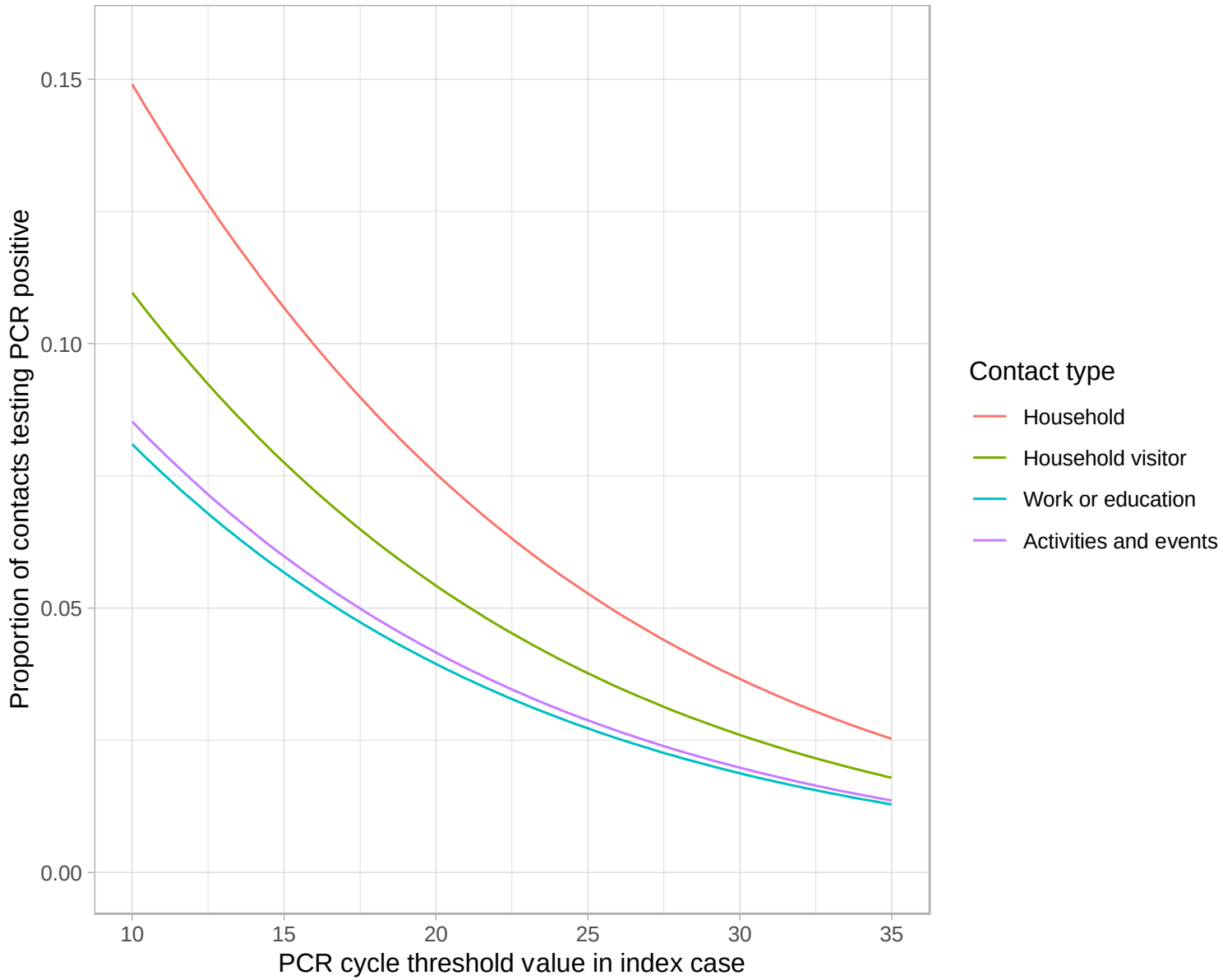


Throat swabs place in 3ml of Buffer

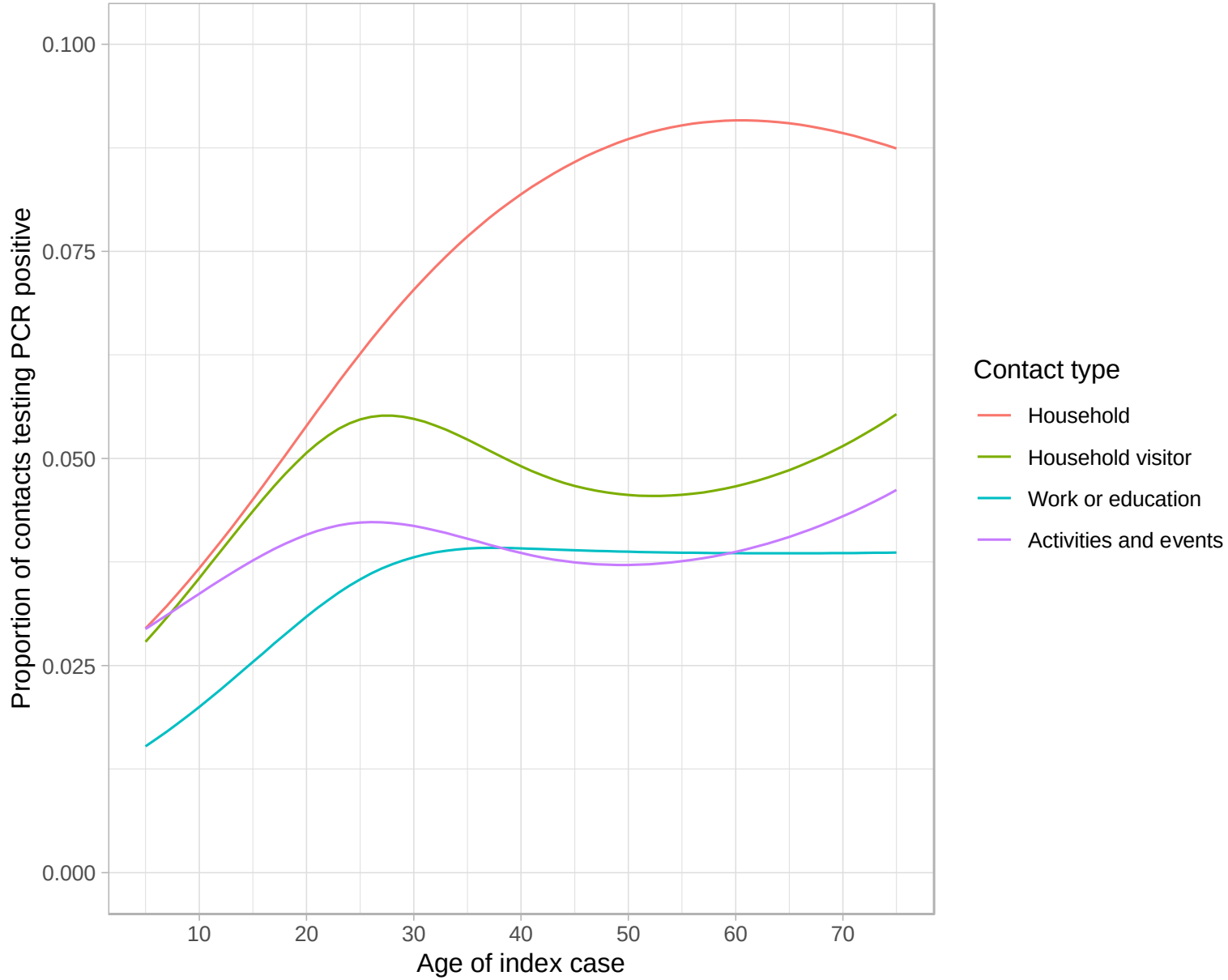
What is the relationship between Viral Load and Infectiousness?

- Biologically likely
- Clear relationship between viral load and culturable virus
- We obtained direct evidence of the relationship between viral load and infectiousness using Test and Trace Data.
- The viral load of the index case was shown to predict the probability that their contacts were infected. 400,000 contact-case pairs were studied
- Results were adjusted for age, demography and types of contact
- Main limitation: no allowance for possible 3rd party transmission

Lee LYW et al Clin Inf Dis (2022) 74 404-415

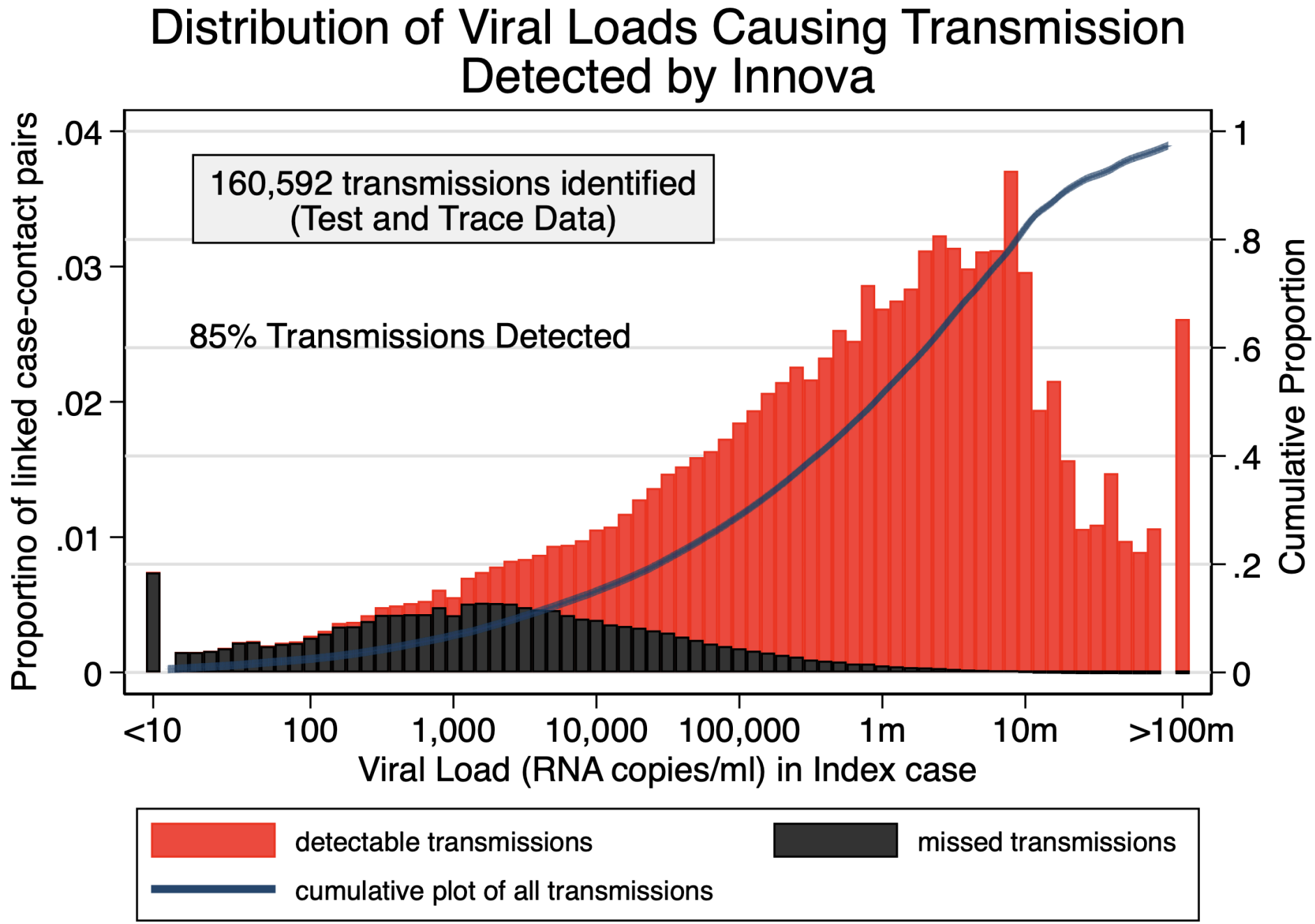


Relationship between PCR cycle threshold (Ct) value in cases and the proportion of their contacts with a PCR positive result, by contact event type. Values are plotted after adjustment for age (set to the median value, 33 years), diagnostic laboratory (set to Milton Keynes), ethnicity (set to white). The shaded area indicates the 95% confidence interval.

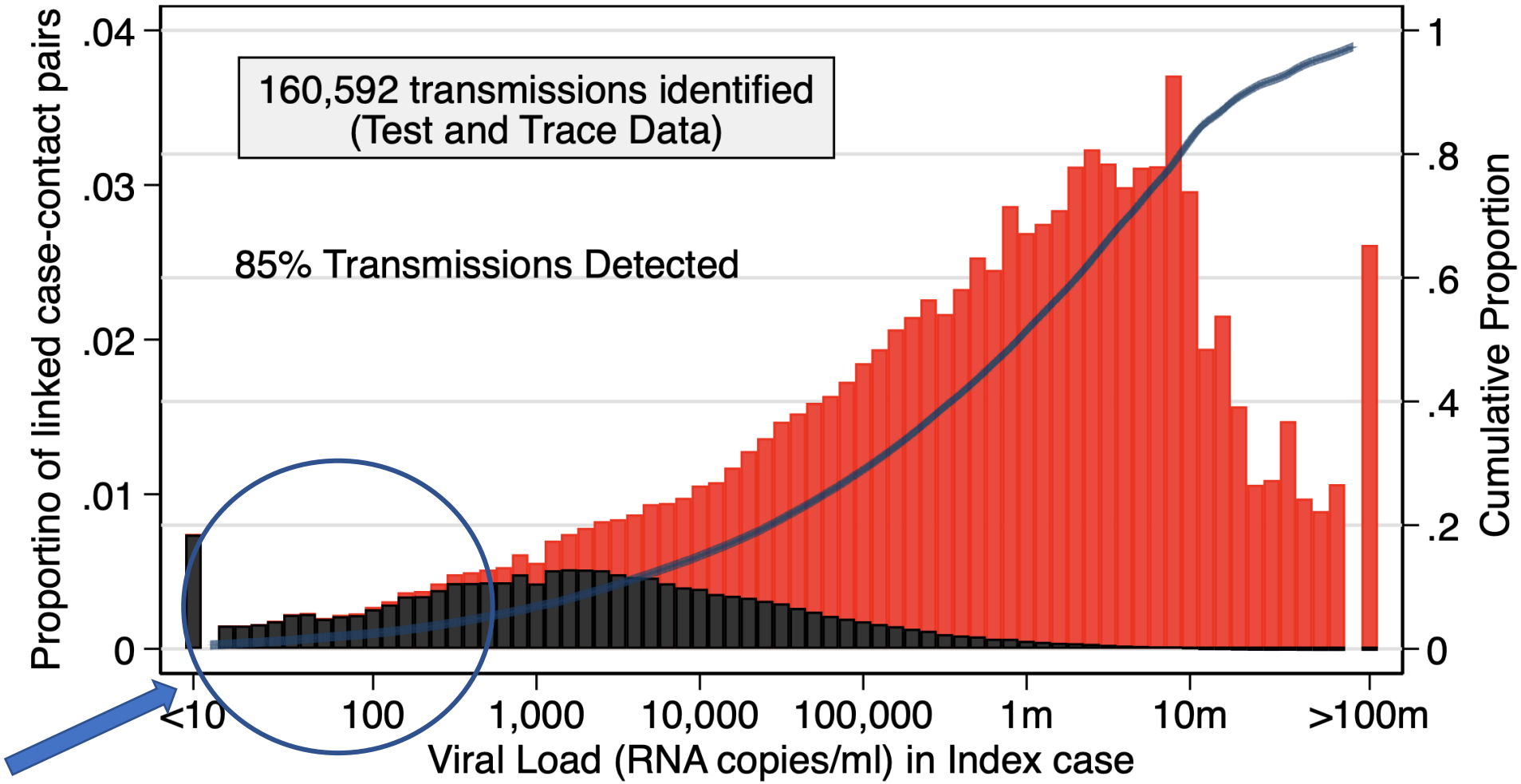


Relationship between case age and the proportion of their contacts with a PCR positive result, by contact event type.

Values are plotted after adjustment for Ct value (set to the median Ct value, 20.2), diagnostic laboratory (set to Milton Keynes), ethnicity (set to white). Age is fitted as a 4-knot spline with an interaction between age and contact event type. The shaded area indicates the 95% confidence interval.


Distribution of Viral Loads Causing Transmission Detected by Innova
160,592 transmissions identified (Test and Trace Data)
85% Transmissions Detected
Proportino of linked case-contact pairs
.04
.03
.02
.01
0
Cumulative Proportion
1
.8
.6
.4
.2
0
<10
100
1,000
10,000
100,000
1m
10m
>100m
Viral Load (RNA copies/ml) in Index case
detectable transmissions
missed transmissions
cumulative plot of all transmissions

Distribution of Viral Loads Causing Transmission Detected by Innova

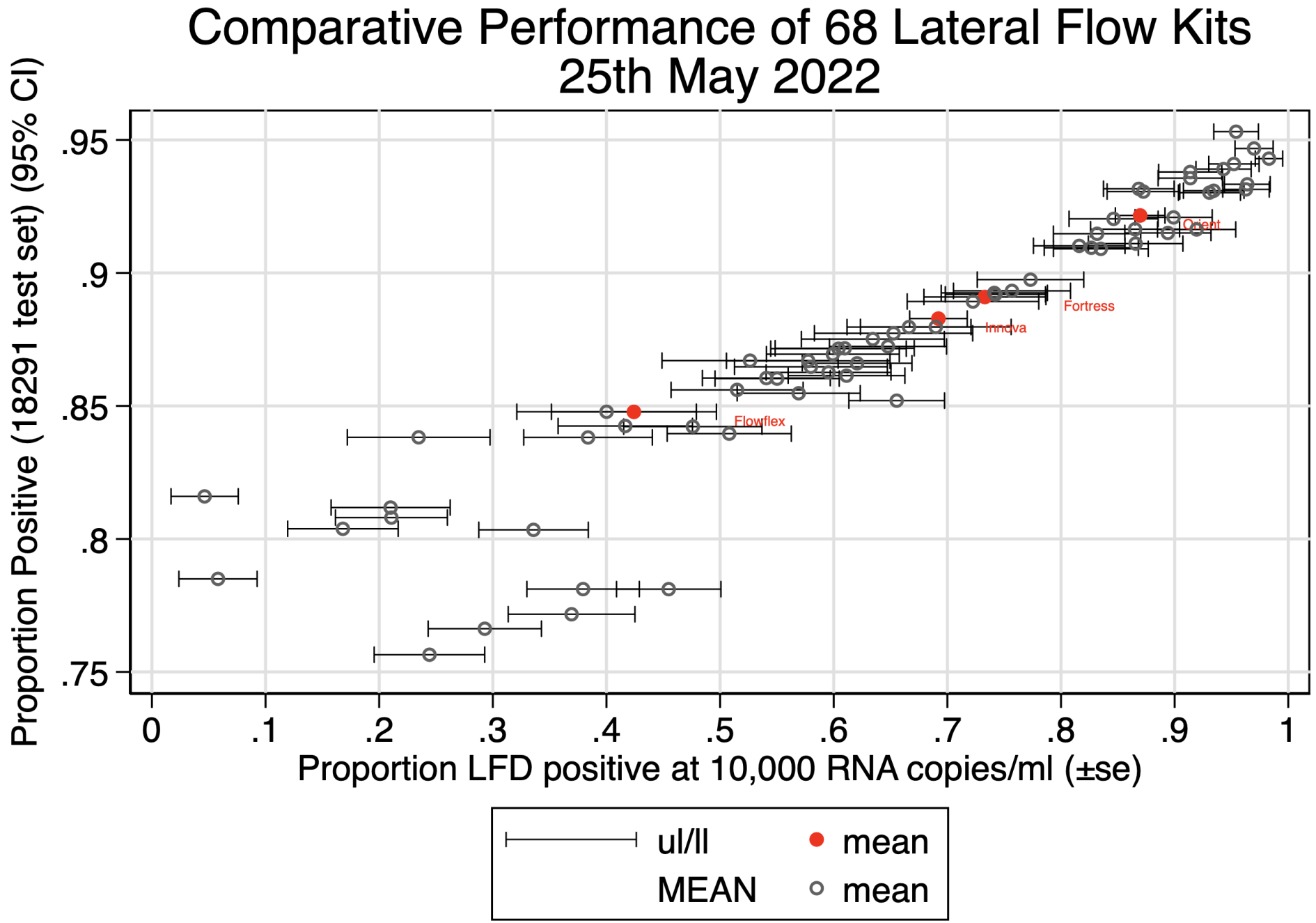


? Due to 3rd Party Transmission

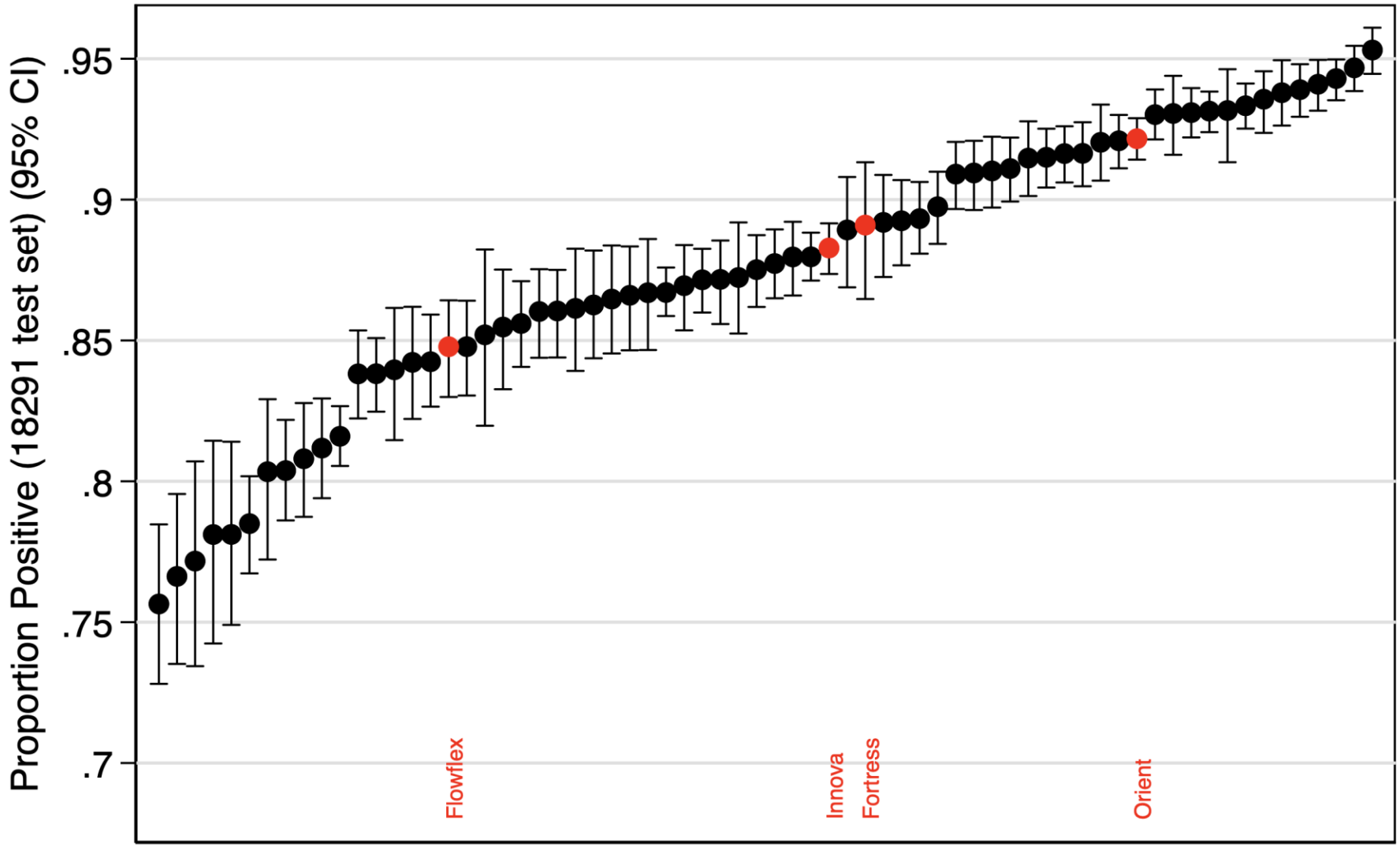
How to compare performance of different Lateral Flow Kits

- The logistic regression model is applied to the viral loads of about 18,291 cases of positive individuals attending test and trace. This is used to assess the overall sensitivity of the test.
- The 18,291 individuals were consecutive individuals who were shown to infect their named contacts (using test and trace data).

NB Adjustment is made to allow for the different volumes of buffer used for PCR (3ml) and lateral flow tests (6 drops about 200ul).


Comparative Performance of 68 Lateral Flow Kits
25th May 2022
Proportion Positive (18291 test set) (95% CI)
.95
.9
.85
.8
.75
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1
Proportion LFD positive at 10,000 RNA copies/ml (±se)
Orient
Fortress
Innova
Flowflex
ul/ll
MEAN
mean
mean

Performance of 68 Lateral Flow Kits (25thMay 2022)



Fortress and Orient are probably identical

Do LFD Detect transmission in Real World Testing

- Randomized trial of 200 schools comparing quarantine with daily lateral flow tests.

Can LFD tests be used to release individuals from quarantine?

Daily testing for contacts of individuals with SARS-CoV-2 infection and attendance and SARS-CoV-2 transmission in English secondary schools and colleges: an open-label, cluster-randomised trial

Bernadette C Young, David W Eyre*, Saroj Kendrick, Chris White, Sylvester Smith, George Beveridge, Toby Nonnenmacher, Fegor Ichofu, Joseph Hillier, Sarah Oakley, Ian Diamond, Emma Rourke, Fiona Dawe, Ieuan Day, Lisa Davies, Paul Staite, Andrea Lacey, James McCrae, Ffion Jones, Joseph Kelly, Urszula Bankiewicz, Sarah Tunkel, Richard Ovens, David Chapman, Vineta Bhalla, Peter Marks, Nick Hicks, Tom Fowler, Susan Hopkins, Lucy Yardley, Tim E A Peto*

Summary

Background School-based COVID-19 contacts in England have been asked to self-isolate at home, missing key educational opportunities. We trialled daily testing of contacts as an alternative to assess whether this resulted in similar control of transmission, while allowing more school attendance.

Lancet 2021; 398: 1217–29
Published Online
September 14, 2021
https://doi.org/10.1016/

Conclusion

Daily contact testing of school-based contacts was non-inferior to self-isolation for control of COVID-19 transmission, with similar rates of symptomatic infections among students and staff with both approaches.

Control Methods against Covid19
LFD

Universal Precautions

- Decrease rate of transmission from all infectious individuals (background)
  - Universal social distancing
  - Use Face marks / hand washing
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Quarantine of individuals at 'high risk' of being infectious

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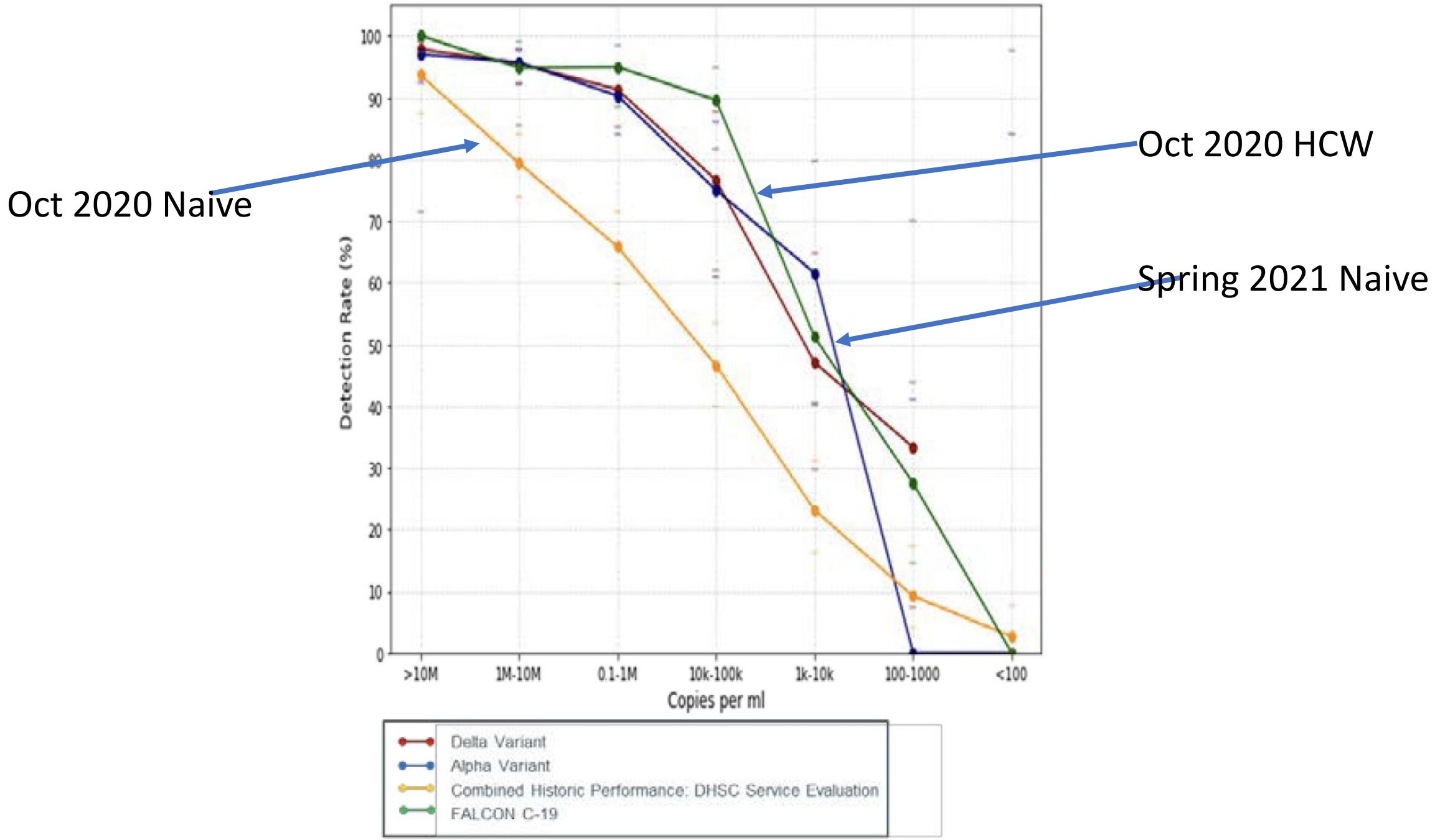
  - Quarantine PCR+ at the time of onset of covid symptom 5
- *Positive Lateral flow antigen – screening test* *c<5*

Follow up Studies

- Porton checks all LFD kits with new variants as they emerge
- Test and Trace have undertaken repeated studies to check performance
  - Population performance is getting better over time

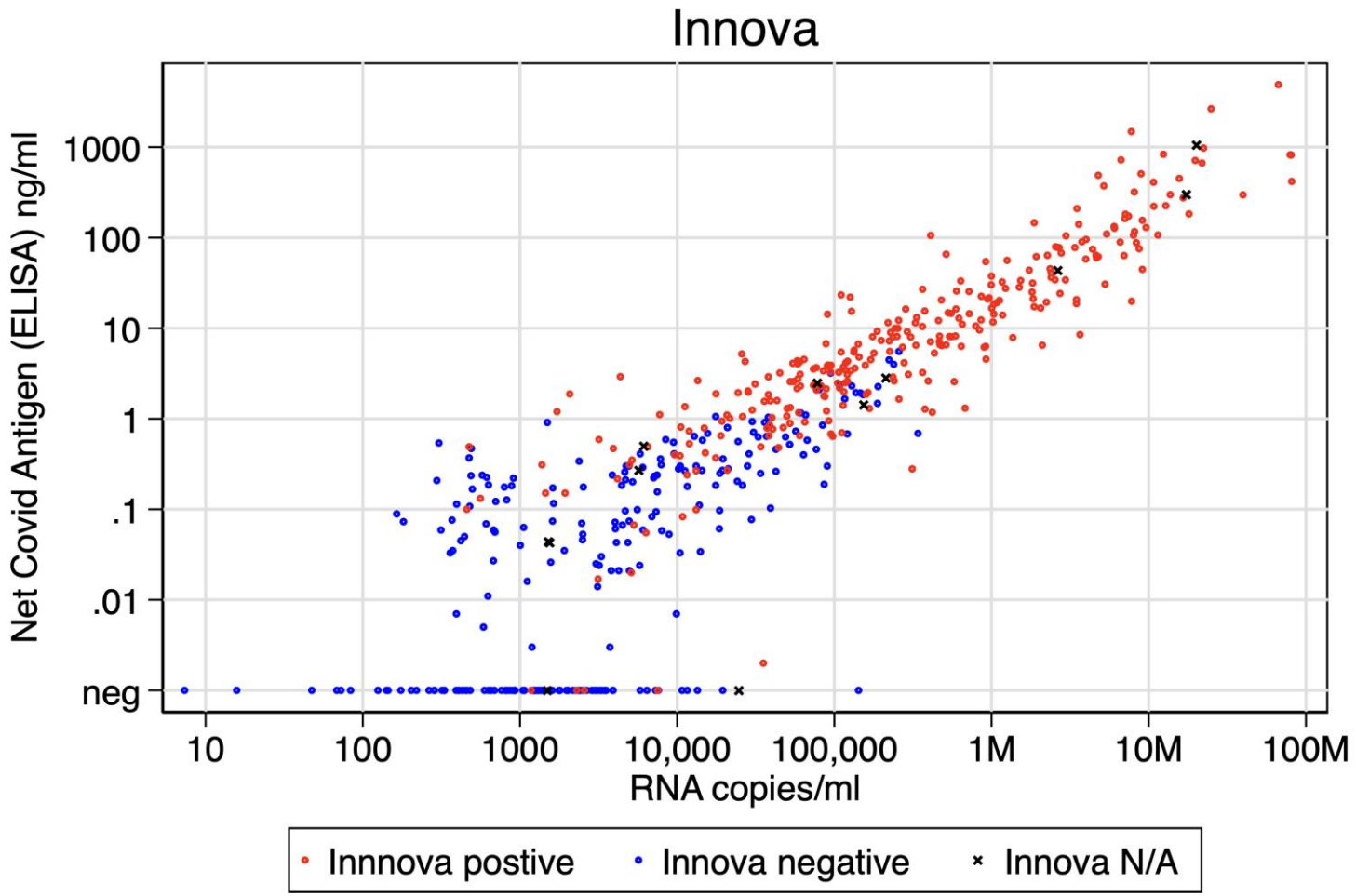

Oct 2020 Naive
Oct 2020 HCW
Spring 2021 Naive
Detection Rate (%)
100
90
70
60
50
40
30
20
10
0
>10M
1M-10M
0.1-1M
10k-100k
1k-10k
100-1000
<100
Copies per ml
Delta Variant
Alpha Variant
Combined Historic Performance: DHSC Service Evaluation
FALCON C-19

Follow up Studies

- Porton checks all LFD kits with new variants as they emerge
- Test and Trace have undertaken repeated studies to check performance
  - Population performance is getting better over time
  - Self testing compared to assisted testing (equivalent)
  - Nasal swabs v Throat swabs (Similar).
  - Large variation of viral loads between two swabs taken from the same individual at same time.
  - Different variants (So far all are detectable)

The infectiousness of LFD/PCR discordants are not known.

More discordants in asymptomatic individuals.



Summary

- Lateral Flow Test are good at detecting antigen
- If positive individuals quarantine on day of test, >85% of onward transmissions can probably be averted.
- Infectiousness of LFD/PCR individuals not known
- LFD have proved much more popular than expected.
  - Most individuals are happy to self-swab their nose and throat
  - Millions of individuals have been detected who have quarantined.