Using trained dogs and organic semi-conducting sensors to identify asymptomatic and mild SARS-CoV-2 infections.

Claire Guest[†], Sarah Y Dewhirst[†], David J Allen, Sophie Aziz, Oliver Baerenbold, John Bradley, Unnati Chabildas, Vanessa Chen-Hussey, Samuel Clifford, Luke Cottis, Jessica Dennehy, Erin Foley, Salvador A Gezan, Tim Gibson, Courtenay K Greaves, Immo Kleinschmidt, Sébastien Lambert, Anna Last, Steve W Lindsay, Steve Morant, Josephine E A Parker, John Pickett, Billy J Quilty, Ann Rooney, Manil Shah, Mark Somerville, Chelci Squires, Martin Walker, James G Logan, on behalf of COVID Dogs Research Team [††]

[†]Joint first authors

[†] Members are listed in appendix 1 pp 2-4

Department of Disease Control, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK (Prof J G Logan PhD, Prof S W Lindsay PhD); ARCTEC, Chariot Innovations Ltd, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK (Prof J G Logan PhD, SY Dewhirst PhD, J E A Parker PhD, V Chen-Hussey PhD, E Foley MSc, C Squires BSc, U Chabildas MRes, M Shah MSc, C K Greaves MSc, J Dennehy MSc, Dr S A Gezan PhD); Medical Detection Dogs, Great Horwood, Milton Keynes, UK (C Guest MSc, S Morant PhD, S Aziz MSc, M Somerville); Department of Biosciences, Durham University, Durham, UK (Prof S W Lindsay PhD); Clinical Research Department, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK (A Last PhD); MRC International Statistics and Epidemiology Group, London School of Hygiene and Tropical Medicine, London, UK (Prof I Kleinschmidt PhD, J Bradley PhD); Department of Infectious Disease Epidemiology, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK (S Clifford PhD, B J Quilty MSc); Department of Medical Statistics, London School of Hygiene and Tropical Medicine, London, UK (O Baerenbold PhD); School of Chemistry, Cardiff University, Cardiff, UK (Prof J Pickett PhD); Lomond Veterinary Clinic Suffolk Street, Helensburgh, Argyll & Bute, UK (A Rooney MSC); Hampden Veterinary Hospital, Aylesbury, Buckinghamshire, UK (L Cottis BVMS); Department of Infection Biology, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK (DJ Allen PhD); RoboScientific Ltd, Wisbech Road, Littleport, Cambridgeshire, UK (T Gibson PhD); Department of Pathobiology and Population Sciences, Royal Veterinary College, University of London, Hatfield, UK (M Walker PhD, S Lambert PhD)

Correspondence to:

Prof James Logan, Department of Disease Control, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK

James.Logan@lshtm.ac.uk

Summary

Background A rapid, accurate, non-invasive diagnostic screen is needed to identify people with SARS-CoV-2 infection. We investigated whether organic semi-conducting (OSC) sensors and trained dogs could distinguish between people infected with asymptomatic or mild symptoms, and uninfected individuals, and the impact of screening at ports-of-entry.

Methods Odour samples were collected from adults, and SARS-CoV-2 infection status confirmed using RT-PCR. OSC sensors captured the volatile organic compound (VOC) profile of odour samples. Trained dogs were tested in a double-blind trial to determine their ability to detect differences in VOCs between infected and uninfected individuals, with sensitivity and specificity as the primary outcome. Mathematical modelling was used to investigate the impact of bio-detection dogs for screening.

Results 3,921 adults were enrolled in the study and odour samples collected from 1097 SARS-CoV-2 infected and 2031 uninfected individuals. OSC sensors were able to distinguish between SARS-CoV-2 infected individuals and uninfected, with sensitivity from 98% (95% CI 95-100) to 100% and specificity from 99% (95% CI 97-100) to 100%. Six dogs were able to distinguish between samples with sensitivity ranging from 82% (95% CI 76-87) to 94% (95% CI 89-98) and specificity ranging from 76% (95% CI 70-82) to 92% (95% CI 88-96). Mathematical modelling suggests that dog screening plus a confirmatory PCR test could detect up to 91% of SARS-CoV-2 infections, averting up to 2·2 times as much transmission compared to isolation of symptomatic individuals only.

Interpretation Our findings demonstrate that people infected with SARS-CoV-2, with asymptomatic or mild symptoms, have a distinct odour that can be identified by sensors and trained dogs with a high degree of accuracy. Odour-based diagnostics using dogs and/or sensors may prove a rapid and effective tool for screening large numbers of people.

Funding Department of Health and Social Care of the UK Government, The National Institute for Health Research, Durham University and charitable donations.

Research in context

Evidence before this study

We searched MEDLINE and Web of Science using the terms 'SARS-CoV-2' and 'COVID-19' with 'dogs' or 'canine' in articles published between Jan 1, 2019 and April 21, 2021. Seven proof-of-concept studies suggest that trained dogs are able to detect symptomatic cases of people with SARS-CoV-2 by their odour, although all have important limitations.

To date, studies have used axillary sweat samples, respiratory secretions, saliva and urine samples as odour sources real-time reverse transcription-polymerase chain reaction (RT-PCR) to confirm whether an individual was infected with SARS-CoV-2 or not. These proof-of-concept studies were generally of low or medium quality and only four are published in peer-reviewed journals. Common limitations include: (1) collecting odour samples from a small number of infected and uninfected individuals, (2) collecting samples from hospitalised patients, who are likely to have severe disease, (3) using virus inactivated samples which will smell different to normal samples, (4) excluding uninfected people with cold-like symptoms, thus being unable to demonstrate a SARS-CoV-2-specific odour, (5) training and testing dogs on samples from a small number of human subjects, making it possible that the dogs memorise specific samples rather than being able to differentiate between samples, (6) always including a positive specimen in a test line, so the dog identifies the 'odd' sample, making it impossible to estimate sensitivity and specificity (known as a forced choice paradigm), (7) not reporting according to STARD criteria, (8) insufficient detail to assess quality of testing, (9) not testing the dogs on novel samples in a double-blind fashion and (10) inappropriate statistical analysis.

We also searched MEDLINE and Web of Science using the terms 'SARS-CoV-2' and 'COVID-19' with 'sensors' or 'electronic noses' or 'odour' in articles published between Jan 1, 2019 and April 21, 2021. A number of reviews have been published on the use of sensor technology for SARS-CoV-2 diagnosis. For example, a wearable device was able to detect abnormal resting heart rates of people infected with SARS-CoV-2 often in advance of symptoms infections. Similarly, a novel AI framework using smartphones with camera sensors, inertial sensors, microphone and fingerprint scanning (temperature) technology has been developed which aimed to aid doctors and medical personnel to rapidly diagnose SARS-CoV-2 infection. Both technologies have limitations due to affordability of these devices, as well as age-group bias towards younger groups.

Electronic noses are a non-invasive electronic sensor technology which detect odours, which could be used as a screening tool. These devices have a wide range of practical applications, such as detecting plant and human diseases and food spoilage, and have shown high efficacy in detecting chronic respiratory conditions such as chronic obstructive pulmonary disease and asthma, as well as gastrointestinal disease. There is a difference in breath odour between infected and uninfected SARS-CoV-2 patients. Two studies collecting breath from patients via an electronic nose, or a device composed of a nanomaterial-based hybrid sensor array distinguished between infected and uninfected patients. We found no published papers on organic semi-conducting (OSC) sensors, and changes in body odour associated with SARS-CoV-2.

Added value of this study

To our knowledge, this study is the first to assess whether trained dogs can distinguish between the odour of people infected with SARS-CoV-2 and those who are uninfected, in a

randomised double-blind trial, where trainer and monitor were unaware of the study group for each sample, and with a sufficiently high number of dogs and individuals donating samples. We are confident that the dogs identified a specific odour signature associated with infection with SARS-CoV-2, as they were tested double blind on samples that had not been used during training. Unlike in most previous studies, we also included asymptomatic and mild cases of SARS-CoV-2 and demonstrate that dogs can identify these individuals, including some with extremely low virus titres, as suggested by high Ct values using real-time RT-PCR. The presence of a distinct SARS-CoV-2 body odour was confirmed using OSC sensors. Mathematical modelling demonstrated that dog screening plus a confirmatory RT-PCR test could effectively detect more cases, resulting in higher levels of transmission averted, compared to isolation of symptomatic individuals only or lateral flow test plus RT-PCR.

Implications of all the available evidence

This study demonstrated that there is a distinct body odour associated with asymptomatic and mild SARS-CoV-2 infections, and that trained dogs and OSC sensors are able to identify this odour with a high degree of accuracy. Modelling showed that trained bio-detection dogs could be used at ports-of-entry or other sites with large numbers of people and should be considered as a new rapid screening tool.

Introduction

To control the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which causes COVID-19, it is essential to rapidly and accurately screen infected individuals within communities, at ports-of-entry and other places where large numbers of people congregate. Although quarantining and testing by reverse transcription-polymerase chain reaction (RT-PCR) is done at some ports-of-entry, these strategies are slow, costly and not-sustainable for long periods. High-throughput screening of inbound passengers is a vital step in preventing onward transmission, whist allowing large numbers of people to travel and economies to open. As new variants emerge,[1,2] the need for rapid and sensitive screening methods is increasingly important to ensure border biosecurity, instil confidence in people to travel and, re-opening and re-invigorate economies which are dependent on travel or congregation of large numbers of people.

Current testing methods for SARS-CoV-2 are unsuitable for rapid screening of large numbers of people, such as those found in airports and other public venues, due to low sensitivity and/or delays in the return of test results.[3] Real-time RT-PCR testing remains the gold standard in SARS-CoV-2 diagnostics, but is impractical for rapid screening due to long turn-around times and a necessity for repeat testing due to false-negatives.[4] Real-time RT-PCR may also detect residual or degraded viral RNA past the point an individual is infectious.[5] The Innova SARS-CoV-2 Antigen Rapid Qualitative Test, a lateral flow test (LFT) may detect individuals with viral loads high enough to cause onwards transmission (83% sensitivity)[6] or those with culturable virus (98% sensitivity)[7] however, may fail to detect lower viral loads.[8] From a practical point of view, real-time RT-PCR and LFT testing may be unsuitable for mass screening since they are invasive, time consuming and costly.

There is evidence that infections with respiratory viruses produce distinct odour signatures that are pathogen specific and may be associated with an odour that could be used for disease detection.[9,10,11,12] Using trained dogs or electronic sensors to identify people infected with SARS-CoV-2 by their odour, might prove to be a tractable method for mass screening passengers since they are non-invasive, able to screen people rapidly in real time,[13] are low cost and potentially scalable. Bio-detection dogs could be used at places where rapid screening of a large number of people is required including ports-of-entry, train stations, sporting venues and places of work.[13]

Bio-detection dogs are increasingly deployed as an efficient, reliable, and mobile diagnostic tools to recognise volatile biomarkers contained in human breath, skin, and urine produced by specific diseases and chronic health conditions.[14,15,16,17] Dogs have an extraordinary olfactory capability with odour detection 10,000-100,000 times higher than an average person, and the dog's lower limit of detection of VOCs is one part per trillion.[18] Several pilot studies suggest that dogs can detect hospitalised patients with SARS-CoV-2 in Colombia, [19] France, [20] Germany, [21,22] Iran,[23] Lebanon, [20] United Arab Emirates[24] and USA[25] with a high sensitivity and/or specificity. These studies, however, had a number of limitations (see evidence before this study).

Here, we aimed to assess whether there is a specific odour associated with infection with SARS-CoV-2, and whether trained dogs and organic semi-conducting (OSC) sensors can distinguish between the odour of uninfected individuals (real-time RT-PCR negative) and infected individuals (real-time RT-PCR positive) who are displaying mild symptoms or are asymptomatic (Appendix 2 pp 5). Modelling was also done to investigate the likely impact of dogs as part of a testing strategy.

Methods

Study design and participants

Participants were recruited from Jul 02, 2020 to Mar 01, 2021 in Great Britain using phone calls, text-messages, emails, leaflets/posters, videos, social media and press articles. The target sample size was 325 SARS-CoV-2 real-time RT-PCR positive (henceforth abbreviated as RT-PCR, infected) participants and at least 675 SARS-CoV-2 RT-PCR negative (uninfected) participants. National Health Service (NHS) staff and their household members were recruited through 23 NHS hospital sites and members of the public recruited via an ARCTEC/LSHTM call centre and Agile Lighthouse (part of NHS Test and Trace). SARS-CoV-2 screening took place in study hospitals, testing centres or remotely through home testing kits.

Individuals were included in the study if they met all the following criteria: (1) due to have a SARS-CoV-2 swab test or had a test in the previous 72 h, (2) aged ≥ 16 years, (3) had suspected mild or moderate, but not severe, SARS-CoV-2 symptoms and did not require mechanical ventilation or palliative care, or were exposed to SARS-CoV-2 or were an NHS staff member undergoing asymptomatic screening, or a household member of NHS staff, (4) no previous laboratory confirmed SARS-CoV-2, (5) willing and able to wear a face mask for 3 h, nylon socks and a shirt for 12 h, (6) provided access to or a copy of their swab test result, and (7) able and willing to provide written informed consent. Samples from participants who were SARS-CoV-2 RT-PCR positive were placed in the infected sample group and SARS-CoV-2 RT-PCR negative were placed in the uninfected sample group.

This study was done in accordance with the recommendations for physicians involved in research on human participants adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions. This study received full approval from the Health and Safety Executive (ref:CBA1.654.20.1, 16-Jun-21), London School of Hygiene and Tropical Medicine Ethics Committee (ref:22159, 22-Jun-20) and Animal Welfare Ethics Review Board (ref:2020-06 19-Jun-20), Department of Biosciences Ethics Committee, Durham University (19-Jun-20), the Health Research Authority (ref:284221, 17-Jun-20), and relevant NHS trust Research Ethics Committees. The study was registered on clinicaltrials.gov (ID: NCT04509713).

Procedures

Samples of breath odours were collected through participants wearing a single-use protective disposable face mask (Fisher Scientific, UK) for 3 h. Skin odours were collected through participants wearing a pair of nylon ankle socks (Retail Premium Grade Try-on Socks, Blue Box Socks, UK) and (t-)shirts, made from natural and synthetic materials, for 12 h. After collection, the odour samples were individually wrapped in aluminium foil and packaged in separate labelled polythene bags by the participant. At the point of return, collected samples were stored frozen ≥ -20^{o}C.

Participants were followed up on return of samples and 14 days after sample collection, to provide details of ill health. Adverse events and serious adverse events in study participants, delegates and dogs were recorded.

Chemical analysis

VOCs from socks collected from 27 asymptomatic or mild symptomatic SARS-CoV-2 infected participants and 27 SARS-CoV-2 uninfected participants were analysed over two

days, using a Model 307B VOC analyser (RoboScientific Ltd, UK) fitted with an array of 12 OSC sensors chosen to be sensitive to the VOCs most likely associated with SARS-CoV-2, in this case, ketone and aldehyde compounds [26] (Appendix 3 pp 6).

Dog training and testing

The methodologies and results for dog training are described in Appendix 3 (pp 6-7) and 4 (pp 11-12) respectively. Here, we summarise the methodology used for double-blind testing.

Sock samples from 200 asymptomatic or mild symptomatic SARS-CoV-2 infected individuals and 200 SARS-CoV-2 uninfected individuals, grouped by sex, age and ethnicity, were used to determine the dogs' diagnostic accuracy. Each sock sample was cut into four pieces, each approximately 80 x 20 mm^2, and sealed individually in vented vials (T-mini jar 43 ml, 43 mm diameter, Pattesons Glass Ltd, UK, covered with a clean nylon sock and sealed with a metal cap) to prevent direct contact by the dog and their handler with the samples, and stored at -20°C before use.

Following the training phase, six of seven trained dogs were deemed suitable for the double-blind testing (Appendix 3 pp 7-9). The dogs were tested using a stand system which held the glass vial containing the sock sample. Testing required an 'infected/uninfected' decision on each sample. Each sample was presented to each dog once, with a maximum of three explorations allowed at the trainer's discretion. Computer software, MDD-Olfactory Performance Recording Application (OPRA), was used to randomly assign odour samples to stands. Therefore, the dogs that worked the three-stand system could have any combination of samples (all infected, all uninfected, or any combination of infected and uninfected) in three positions. A blinded handler, positioned behind a one-way screen (so that the dog could not receive visual prompts from the handler), tasked the dog to search the stands, off lead.

Statistical analysis

Sample size (Appendix 3 pp 10) was based on the requirement to estimate sensitivity and specificity with sufficiently high precision. Statistical analyses were done in R version 4.0.3 (chemical analysis) and Stata version 16 (double-blind testing).

Principal components analysis (PCA) was used to identify potential differences in the odours from infected and uninfected individuals, and to obtain biplots (Appendix 3 pp 10). In addition, linear discriminant analysis (LDA) was done to determine sensitivity and specificity based on cross-validation. Each day's evaluation was assessed separately.

Sensitivity and specificity were calculated separately for each dog, assuming PCR was the gold standard. A Bayesian latent class analysis[27] that allows for imperfect sensitivity and specificity of PCR was also carried out (Appendix 3 pp 10 and Appendix 4 pp 13). The Bayesian analysis had weakly informative priors for the sensitivity and specificity of the dogs and PCR. Improvement in the dogs' sensitivity and specificity over time and the association with RT-PCR cycle thresholds (Ct) values and sensitivity were assessed using logistic regression with a linear effect for day of study or Ct value, respectively.

Mathematical modelling

We used a modelling approach, adapted from Quilty and co-workers,[8] to explore the effectiveness of a "Rapid Screen and Test" strategy using dogs plus confirmatory PCR. We compared this to: (1) a baseline scenario of self-isolation of symptomatic individuals only, (2) screening with LFTs followed by confirmatory PCR, and (3) mass screening with PCR

(Appendix 3 pp 8). We simulated RT-PCR Ct trajectories of infected individuals as a proxy of viral load. Effectiveness was quantified as the proportion of cases identified and the ratio of transmission averted compared to baseline (i.e. isolation of symptomatic individuals only). Transmission averted was calculated as the time that an individual would have spent with a Ct less than 30,[28] if they had not been screened and isolated.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Characteristics of study samples

A total of 3,921 participants were recruited (Figure 1). A summary of reported adverse events is in Appendix 4 pp 11. Table 1 shows the characteristics of the samples used in double-blind testing.

Chemical Analysis

The OSC sensor array was able to distinguish between infected and uninfected samples, demonstrating that SARS-CoV-2 has a distinct odour. On both days of testing, with the first two dimensions, PCA showed extremely clear clustering of the samples from infected and uninfected participants (Figure 2). OSC sensors achieved 98% (95% CI 95-100) specificity and 99% (95% CI 97-100) sensitivity on the first day of analysis, and 100% sensitivity and specificity on the second.

Double-blind study to assess sensitivity and specificity of dogs

Dogs were able to identify samples from infected (asymptomatic or mild symptoms) individuals with high accuracy. The highest performing dog achieved 94% (95% CI 89-98) sensitivity and 92% (95% CI 88-96) specificity under double-blind conditions (Table 2). Overall, the six dogs achieved a sensitivity range of 82- 94% and a specificity range of 76-920%. Specificity (p=0·003), but not sensitivity (p=0·650), increased as double-blind testing progressed. There was no evidence for an association between sensitivity and virus quantity in samples, measured by Ct value as a proxy of viral load (p=0·801, Figure 3A). The range of observed Ct values was 20·9 to 35·4. The dogs correctly identified B.1.1.7 variant (‘Kent variant’) samples 79% of the time (38/48 presentations). There was no evidence sensitivity to the B.1.1.7 variant was lower (p=0·392) despite the dogs having not been trained using this variant. How sensitivity and specificity was affected by sample characteristics is shown in Appendix 4 pp 14.

Mathematical modelling

Modelling indicated that a strategy using dogs, plus RT-PCR for those people indicated as positive by dogs, detected 91% (95% credible interval: 85% to 96%; Figure 3E) of cases resulting in 2·24 (95% credible interval: 1·84 to 2·88; Figure 3F) times as much transmission averted compared to isolation of symptomatic individuals only. In comparison, mass testing with RT-PCR alone detected 100% of cases and the amount of transmission averted was 2.36

(95% credible interval: 1.95 to 3.07), demonstrating the performance of dogs was similar to RT-PCR. Screening using dogs was superior to using LFTs for all assumptions on dog sensitivity (varied between 80% and 90%) but is dependent on the assumed sensitivity of RT-PCR for low viral loads (Figure 3E and 3F). The sensitivity of RT-PCR at low viral loads, as occurs during the early or late stage of infection or if true asymptomatic, is not well established in the literature, and therefore, we modelled all testing scenarios (LFT + RT-PCR, dogs + RT-PCR and RT-PCR alone) with 100% sensitivity of RT-PCR up to either 35 or 40 Ct for comparison. It is noteworthy that if RT-PCR has, in practice, no sensitivity in the Ct 35-40 range, even mass testing using RT-PCR would detect only approximately 64% of infections (Figure 3e). This is because infected individuals have a Ct value between 35 and 40 for approximately one third of the duration of infection (Figure 3C, inset).

Discussion

Our findings demonstrate that trained dogs and OSC sensors can distinguish between the odour of individuals with asymptomatic or mild symptomatic SARS-CoV-2 infection, and uninfected individuals with a high degree of accuracy. Work with the sensors shows that the VOCs associated with SARS-CoV-2 can generate an odour 'fingerprint' as the sensors were tuned to ketone and aldehyde compounds, but may also lead to the development of an OSC sensor device which could be used to screen air from rooms (e.g. classrooms) or aircraft cabins. This would allow the detection of one infected individual within a room or aircraft, allowing rapid and more targeted testing to be done, saving money and time, and reducing onward transmission. The confirmation by VOC analysis that there is a distinct odour between the two groups, and the addition of the tentative identification of the volatile chemicals involved, may also enable the production of training aids for dogs (pseudo-odours), reducing the time spent required to obtain samples for training.

After just six weeks training, six dogs discriminated between odour samples from 200 infected participants and 200 uninfected participants with a sensitivity range of 82-94% and a specificity range of 76-92% compared with the reference test, RT-PCR. In our analysis we adjusted for the RT-PCR being imperfect and recognised that there was a degree of concordance between dogs. Similar values of sensitivity and specificity have been recorded in a number of pilot studies,[20,21,22,23,24,25] although uncertainty in the estimates from these studies is high because of inadequate study design (e.g. low power).

Dogs could be deployed to screen odours collected from individuals, as was done here. This could be used in situations where clothing or skin swabs could be collected and tested. The method used here could also be adapted to screen real people. Our recent work shows that dogs trained in the study readily transition from laboratory-sample testing to identifying people wearing shirts worn by people with SARS-CoV-2 (unpublished). This work is encouraging and suggests that trained dogs will readily identify people infected with SARS-CoV-2 from lines of uninfected people. Specificity improved during testing, with both dog and trainer increasing in confidence, as would likely happen within real-world testing. We postulate that both sensitivity and specificity will improve further in real-world settings, with SARS-CoV-2 positive passengers providing larger and clearer odour profiles than used for this proof-of-concept study.

A relatively narrow range of sensitivity and specificity was apparent between the different dogs tested. In practice, only the highest performing dogs would be deployed. Our results suggest, however, that, in this experimental setting, the dogs had a higher accuracy than the LFT which has a wider range and lower overall sensitivity of between 58-77%.[29] RT-PCR is the gold standard test due to a high sensitivity (97-99%) and specificity (95-99%),[30] but dogs

have a major advantage over both these tests as they are incredibly rapid. Our preparatory work indicates that two dogs could screen 300 people in 30 min, for example, the time it takes to disembark from a plane, and PCR would only need to be used to test those individuals identified as positive by the dogs (Figure 4). This would result in far fewer individuals needing a RT-PCR test, allowing most travellers to continue their onward journey or mass event attendees with little inconvenience. If used at airports, dogs may also serve as a visual deterrent, reducing passengers travelling with false SARS-CoV-2 negative certificates, as has anecdotally been observed with explosive and drug detection dogs serving as a visual deterrent against drugs and explosives being brought to public events.

Mathematical modelling suggested that a "Rapid Screen and Test" strategy, using dogs plus confirmatory real-time RT-PCR of positively identified individuals, could be highly effective in detecting cases and averting transmission. This screening strategy could be used in a variety of targeted settings and scenarios where the greatest impact could be achieved. Our results indicate that dogs outperform LFT (as an alternative rapid screening tool) across sensitivities between 80-90%. An important reason for this is the seeming independence of dog sensitivity and viral load (using Ct as a proxy) which contrasts with the rapid decline in sensitivity for the LFT with increasing Ct. Even if the sensitivity of dogs fell to zero for Ct values greater than 35, they would still perform better than LFTs, due to the extremely low sensitivity of LFTs for Ct values in this range.

Interestingly, we found our estimates of effectiveness to be very sensitive to the performance of RT-PCR in detecting low viral loads (Ct values >35). For example, in the best-case scenario where RT-PCR sensitivity remains high for Ct > 35, the Rapid Screen and Test strategy detects 80-90% of cases. This drops precipitously to 50-60% if the diagnostics cannot (in practice) detect Ct values between 35-40, indicative of low viral loads. Indeed, that RT-PCR alone can detect only approximately 60% of infections if it is insensitive in the Ct 35-40 range, may have implications for the interpretation of prevalence estimates made by random testing of populations. The proportion of time an infected individual spends with low Ct in the 35-40 range is similar for symptomatic and asymptomatic individuals,[29] indicating that adjusting the ratio of asymptomatic and symptomatic individuals will have little impact on the projected performance of Rapid Screen and Test. In future, the modelling approach could be used to evaluate the effectiveness of screening in other contexts, such as public venues, mass events, and domestic travel hubs where case detection could avert potential superspreading events.

Our study has a number of limitations. Firstly, although dogs could be used to screen samples, the real value would be screening people, and we have not yet tested dogs on people infected with SARS-CoV-2. Secondly, our results suggest dogs are able to detect the B.1.1.7 variant, although the sample size was not sufficient for a reliable estimate of sensitivity. In the event that a new variant of SARS-CoV-2 resulted in a different odour profile, trained dogs could be rapidly re-trained to detect the new odour within two days providing odour samples for the new variant are available. Thirdly, there is a possibility that other respiratory viral infections produce similar odour signatures to SARS-CoV-2. This is, however, unlikely given that 26% of uninfected participants in our study displayed classic SARS-CoV-2, cold or flu-like symptoms, and the dogs correctly identified them as uninfected. Additionally, other studies suggest that different viral infections result in distinct odour profiles.[9,10, 11 ,12]

Conclusion

Our study demonstrated that trained dogs and OSC sensors can detect people with asymptomatic and mild SARS-CoV-2 infections by their odour with a high degree of

accuracy under laboratory conditions and should be considered as an additional tool for use in SARS-CoV-2 testing strategies. This diagnostic has the potential to screen large groups of people rapidly, accurately and non-invasively.

Contributors

JL, CG, SWL, AL, JB, IK, DJA, SYD conceived and designed the study. IK, JB planned the statistical analysis. JL led the study. SYD, CS, UC, MS oversaw collection of samples from NHS hospitals. SM, SA generated the random allocation sequence that assigned the samples for testing. SYD, JEAP, EF supervised collection, collation, preparation and storage of samples. SYD, UC, MS implemented quality assurance for the trial. AL, CKG took responsibility for patients in the trial and AR, LC for the health of the dogs. CG, MS, SA trained and tested the dogs. TG screened SARS-CoV-2 VOCs and produced the OSC sensor array. TG, JP carried out the chemical analysis. VCH led the data management team. JB, OB, SAG, TG did the statistical analysis. BJQ, SC, MW, SL, JD performed the mathematical modelling. SWL, SD, JL, EF, JD produced the first draft of the manuscript. All authors read and approved the final manuscript.

Declaration of interests

We declare no competing interests. Medical Detection Dogs is a registered charity in England and Wales No. 1124533 and in Scotland No. SC044434. Tim Gibson is the Chief Scientific Officer of RoboScientific and holds shares.

Data sharing

All data will be archived at ARCTEC (London School of Hygiene & Tropical Medicine). A dataset containing de-identified participant data that support the findings of this article is available from the corresponding author (JL) upon reasonable request and accompanied by IRB approval. Data is available immediately, for 10 years, after which archives may be destroyed. The study protocol is in Appendix 6.

Acknowledgements

We are grateful to the participants who donated the samples, staff in NHS hospitals and testing centres. We thank members of the Trial Steering Committee for their helpful suggestions, time and commitment. The study was supported by a grant from Department of Health and Social Care, UK Government (2020/023), Durham University COVID-19 response fund (RF020929), NIHR Clinical Research network support (IRAS ID 284222) and charitable donations.

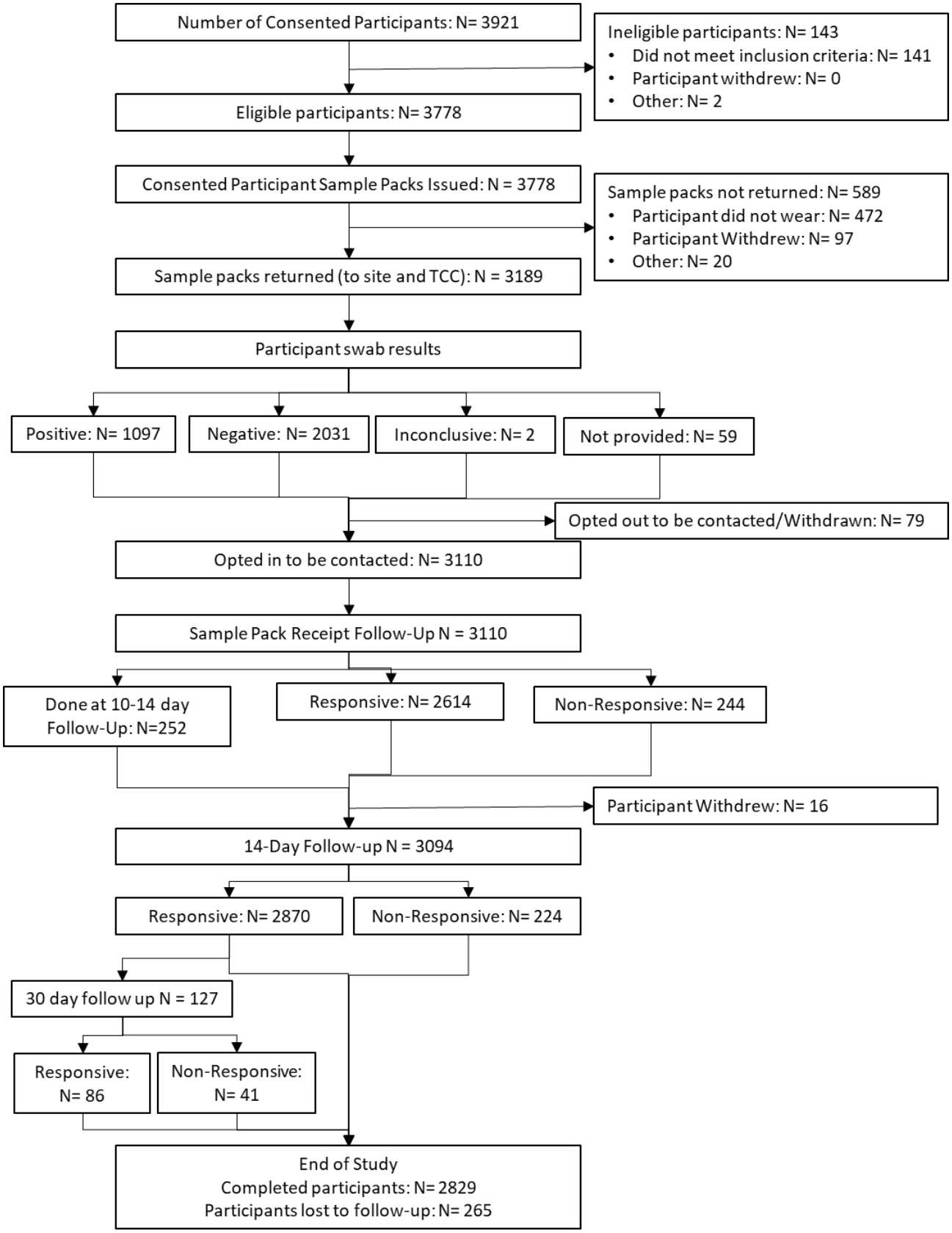

Figure 1: **Flow chart of participants through the study.**

	Infected group (RT-PCR +ve, n=200)	Uninfected group (RT-PCR -ve, n=200)
Source of sample		
ARCTEC/LSHTM call centre & Agile Lighthouse	175 (87·5%)	9 (4·5%)
NHS hospitals	25 (12·5%)	191 (95·5%)
Gender		
Women	147 (73·5%)	155 (77·5%)
Men	53 (26·5%)	45 (22·5%)
Age, years		
16-50	129 (64·5%)	117 (58·5%)
>50	71 (35·5%)	83 (41·5%)
Ethnicity		
White	190 (95·0%)	172 (86·0%)
Asian	6 (3·0%)	4 (2·0%)
Black	1 (0·5%)	1 (0·5%)
Other	3 (1·5%)	3 (1·5%)
Unknown	0 (0·0%)	20 (10·0%)
Symptoms at enrolment		
Classic SARS-CoV-2	148 (74·0%)	41 (20·5%)
Non-classic SARS-CoV-2	52 (26·0%)	159 (79·5%)
Hospital patients	0 (0·0%)	0 (0·0%)
Symptoms at sample receipt at site		
Classic SARS-CoV-2	80 (40·0%)	11 (5·5%)
Non-classic SARS-CoV-2	76 (38·0%)	168 (84·0%)
Unknown	44 (22·0%)	21 (10·5%)

Symptoms after 14 days		
Classic SARS-CoV-2	65 (32·5%)	3 (1·5%)
Non-classic SARS-CoV-2	121 (60·5%)	191 (95·5%)
Unknown	14 (7·0%)	6 (3·0%)

Table 1: **Characteristics of odour samples used for dog testing**

Symptoms at enrolment, at sample receipt at site and 14-day follow-up were categorised as "classic SARS-CoV-2" if fever, cough, or loss or change of smell or taste were reported, and "non-classic SARS-CoV-2" for those who reported no symptoms or where other symptoms were reported, including, shortness of breath, abdominal pain, muscle and joint pain, conjunctivitis or nausea. NHS hospitals: BHAM (1 uninfected), BSDN (1 infected, 12 uninfected), BUCK (47 uninfected), CAWH (9 uninfected), DBTH (14 infected, 4 uninfected), GETH (1 uninfected), JUHL (3 uninfected), KETG (1 infected, 15 uninfected), KMSF (6 uninfected), MACH (2 infected, 1 uninfected), MCRI (1 infected, 8 uninfected), MGPH (18 uninfected), MYSH (29 uninfected), PGHL (3 infected, 1 uninfected), UCLH (2 infected, 2 uninfected), UHCW (1 infected, 4 uninfected), UHMB (16 uninfected), WHAD (14 uninfected). All swabs were processed through routine NHS channels, apart from 1 positive which were carried out through non-NHS testing route (Private hospital).

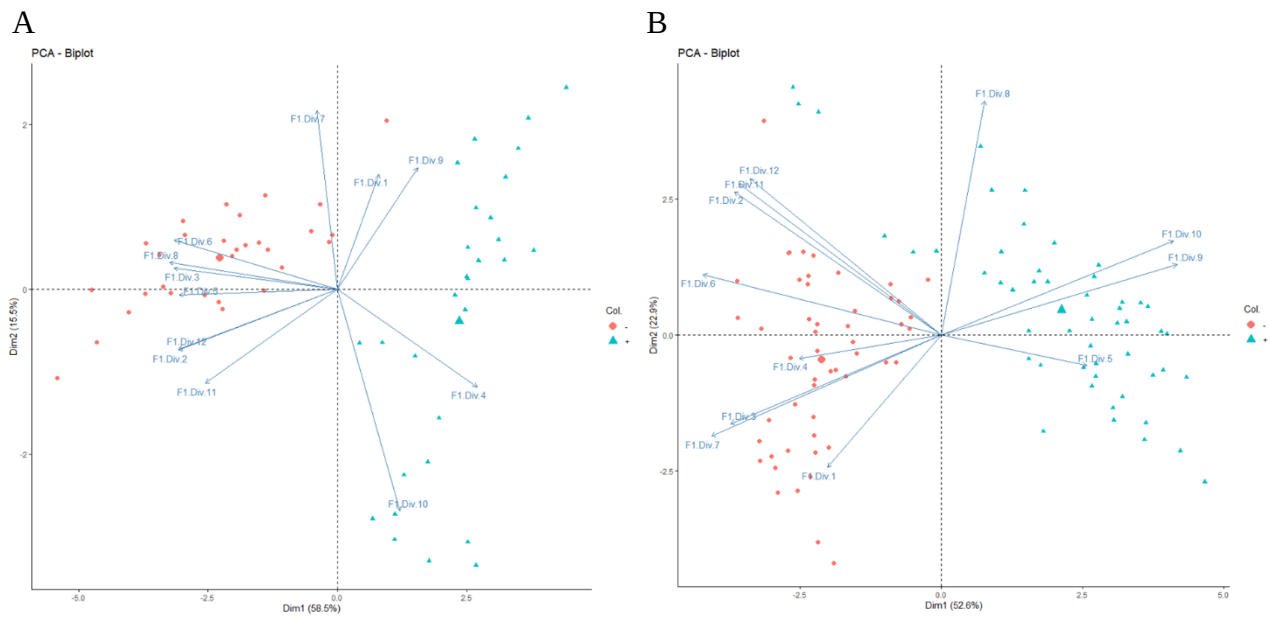

Figure 2: **Principal component analysis of odour samples by organic semi-conducting (OSC) sensors on two different days; (A) Day 1 and (B) Day 2.** Where red circles SARS-CoV-2 infected samples and green triangles are SARS-CoV-2 uninfected odour samples.

	Study group		**Analysis assuming PCR as gold standard**		**Bayesian analysis allowing for imperfect PCR measurements**	
	RT-PCR +ve	RT-PCR -ve	Sensitivity % (95% CI)	Specificity % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)
Asher	115/129	110/132	89·1 (82·9-93·6)	83·3 (76·3-88·9)	90·9 (85·3-95·4)	84·8 (77·9-91·1)
Kyp	172/200	151/200	86·0 (80·7-90·3)	75·5 (69.2-81.1)	88·5 (83·6-92·8)	76·4 (70·3-82·1)
Lexi	172/200	165/200	86·0 (80·7-90·3)	82·5 (76·8-87·3)	90·8 (86·0-94·9)	85·3 (79·9-90·2)
Marlow	157/200	177/200	78·5 (72·4-83·8)	88·5 (83·5-92·4)	82·1 (76·3-87.3)	90·1 (85·4-93·9)
Millie	163/200	161/200	81·5 (75·7-86·4)	80·5 (74·6-85·5)	85·5 (80·1-90·5)	82·6 (76·9-87·6)
Tala	178/200	178/200	89·0 (84·1-92·8)	89·0 (84·1-92·8)	94·3 (89·4-98·0)	92·0 (87·6-95·8)

Table 2: Results of double-blind testing for each trained dog Where data are n/N, CI=confidence intervals.

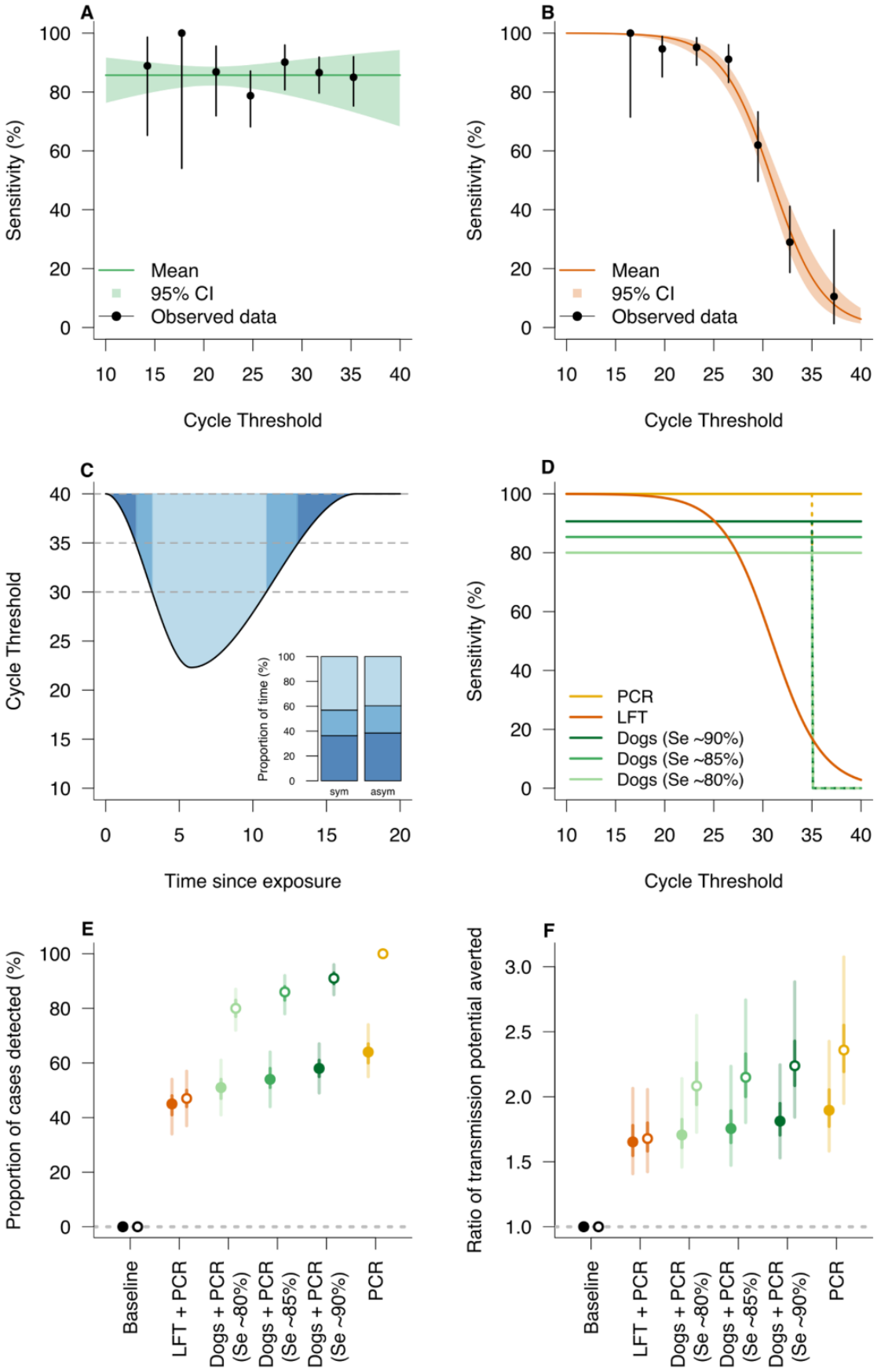

Figure 3: Modelling the effectiveness of a Rapid Screen and Test strategy. The Ct-dependent sensitivity was estimated by fitting a logistic regression model to the results of the

double-blind testing (this study) for dogs and to the data presented for the lateral flow test (LFT) in Peto.[28] Results show that sensitivity is independent of Ct for dogs (panel A; p=0·998) whereas sensitivity decreases with increasing Ct values for LFT (panel B; p<0·0001). The cycle threshold (Ct) is considered a proxy for viral load and is repeatedly simulated from a distribution defined by a starting Ct, a peak Ct and a total duration of infection with a random time since initial exposure. Panel C shows the relationship between Ct and time since exposure for a typical symptomatic individual (asymptomatic individuals having 40% shorter duration of infection). Inset panel shows that both symptomatic and asymptomatic individuals have Ct values between 35 and 40 for approximately one third of the duration of infection. The modelled relationship between sensitivity and Ct for PCR, LFT and dogs is shown in panel D. The sensitivity-Ct relationship for dogs (light green line, 80%; green line, 85%; dark green line, 90%) and LFT (orange line) was informed from data as shown in panels (a) and (b). The sensitivity for PCR was assumed to be 100% up to a Ct of 35, either remaining at this level to a Ct of 40 (yellow solid line) or declining to 0% between 35 and 40 (yellow dotted line). This uncertainty of sensitivity between Ct values of 35 and 40 was also considered for the dogs, with different sensitivity estimated from the data of the double-blind testing (green dotted lines) and representing variability in dog performance. The percentage of cases detected by different strategies is shown in panel E, where baseline corresponds to isolation of symptomatic individuals only and PCR corresponds to the (hypothetical) screening of all individuals with PCR. LFT + PCR and Dogs + PCR indicate, respectively, rapid mass screening with LFTs or dogs followed by confirmatory PCR of positively identified cases. The ratio of the transmission averted by these scenarios compared to baseline is shown in panel F. In panels E and F, filled and open points correspond to a Ct detection limit of 35 and 40 respectively.

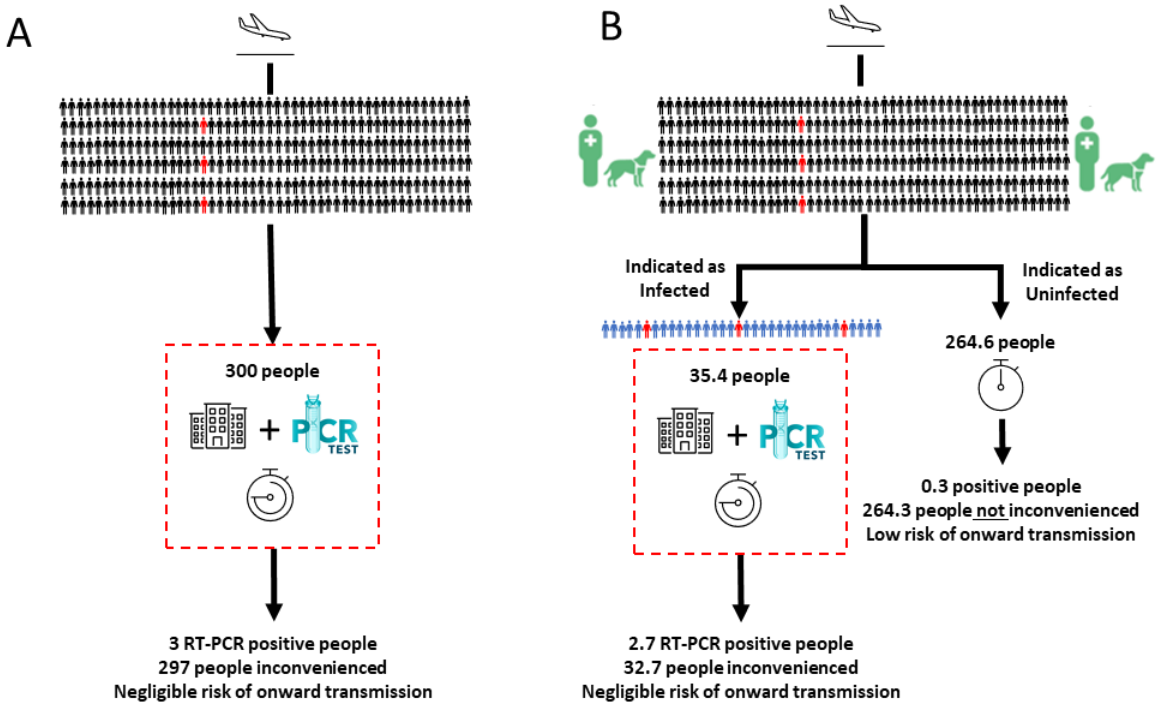

Figure 4: **Exemplar of (A) Current SARS-CoV-2 Strategy and (B) Proposed Rapid Screen and Test Strategy.** Schematic outlining the number of true negatives (black) and true positives (red) and false negatives (blue) as a result of screening people, with 1% SARS-CoV-2 prevalence, followed by confirmatory PCR testing. Assuming 100% sensitivity and specificity of RT-PCR, and 90% sensitivity and 89% specificity of dogs (values chosen to represent the highest performing dogs and subsequently used in the mathematical modelling). 'Inconvenienced' refers to virus-negative passengers required to be in quarantine (red dotted line).

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