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A Two-Phase, Single Cohort Study of COVID-19 Antibody Sera-Surveillance

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Abstract

Objective: The infectious corona virus, COVID-19 has high case mortality in those whom suffer with severe symptoms requiring hospitalisation. A major problem associated with COVID-19 is the spread of infection by a-symptomatic carriers, or those with mild symptoms. We aim to determine the antibody prevalence in a professional Welsh cohort and begin to explore the longevity of COVID-19 antibodies.

Methods: 739 Cardiff Metropolitan University staff members took part in an observational study to determine the prevalence of COVID-19 antibodies in a two-phase, single cohort study. All participants were tested for IgM and IgG antibodies against COVID-19 using a lateral flow detection assay. Venous blood samples from positive participants and a randomly selected negative population were collected to confirm antibody titre, using two gold standard immunoassays, carried out independently by the Specialist Virology Centre, UHW.

Results: 3.65% of the population tested positive for antibodies against COVID-19, with a higher prevalence seen in male participants (5% vs. 2.73% of females). In addition to gender, both pre-existing asthma and age were key determinants in antibody positivity. 78.26% retained antibodies at the 3 months follow up test. 36.36% of females lost antibody positivity between the 3 - and 6 - month time points compared with 8.3% of males. Lateral flow antibody testing was shown to have 96% sensitivity and 95% specificity compared with standard tests.

Conclusion: We conclude that prevalence of COVID-19 antibodies is evident in the asymptomatic population, and in 78.26% of those initially antibody positive prevails at approximately 6 months from perceived time of exposure. Males are 4 times more likely to retain antibodies for longer than females.

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Introduction

COVID-19 is an infectious corona virus, with a high case fatality rate caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified in Wuhan in China, in December 2019[1]. The ongoing mass spread of COVID-19 has been attributed to transmission of respiratory droplets, aerosols and contaminated surfaces [2]. Despite COVID-19 being declared by the World Health Organisation as a public health emergency of international concern, at the end of January 2020, the majority of COVID-19 cases were a-symptomatic or associated with mild symptoms [3]. Some patients have and continue to progress to have severe pneumonia-like symptoms that can require hospitalisation. During the first phase of the COVID-19 pandemic and at the time of initiating this study, the majority of research investigating COVID-19 outcomes and antibody prevalence had focused primarily on patients with severe disease, often those receiving intensive care where mortality rate was as high as 59% (between January and March)[13]. More recently, data has emerged from a number of studies in a-symptomatic cohorts including UK Biobank [4], Imperial [5] and Iceland [6]. Our study focuses on COVID-19 antibody prevalence in a single cohort of largely a-symptomatic participants at two time points, comparing Lateral Flow Testing (LFT) to two clinically applied standard immuno-methods.

After infection with COVID-19, most (but not all) people will develop antibodies against the virus [7,8]. The extent of antibody response and lifetime depends on several factors including the extent of exposure, individual immune system responsiveness and pre-existing conditions [9]. Several high through put lateral flow immunoassays were developed by a number of companies early during the pandemic in response to the crisis and the limited availability of gold standard Polymerase Chain Reaction (PCR) detection of virus. These were recognised to have several advantages including detection of both IgM and IgG antibodies within ten minutes, the wide scale availability, low cost, and their ease of use [14]. These stimulated early limited scale population sera-surveillance to be carried out, such as, in Southampton (545, a-symptomatic healthcare workers) [10] and in the Isle of Wight (1 third of healthcare workers) [15]. The potential for these assays to inform incidence of COVID-19 infection and antibody prevalence locally and nationally was attractive. However, results from these studies found LFT to have lower sensitivity and specificity in practice in comparison to PCR for virus and clearly did not report on current status of viral infection. They were therefore deemed largely inappropriate for wide scale adoption across the asymptomatic population at that time, with high sensitivity PCR capacity being focussed on those individuals who were symptomatic.

Determining antibody prevalence within the community could play an important role in increasing our understanding of the pathophysiology and epidemiology of COVID-19, not only will this allow those people who have developed an immune response to COVID-19 to be identified but serial testing will provide valuable insight into the longevity of antibodies in response to COVID-19 infection. Ultimately, antibody prevalence in conjunction with the T cell adaptive immune response may convey a level of immunity that would help prevent re-infection or reduce COVID-19 disease severity – the basis of vaccination. Recognising the wide scale applicability of LFT and by employing the product with the highest sensitivity and specificity commercially available at the time, we investigated the usefulness of high through - put LFT for antibody measurement and determined the test's sensitivity and specificity by comparing its results to two gold standard, high sensitivity immune assays carried out independently by the Specialist Virology Centre, UHW.

We aimed to determine antibody prevalence in a low risk, a-symptomatic cohort who had been in UK "lockdown" since mid-March, and track these over the ensuing period of uncertainty as we approached and entered a second phase of acute widespread infection. We believe that results from our cohort may be representative of the larger population of professionals, asked to work from home in accordance with Welsh government guidelines March through November.

Methods

All full time Cardiff Metropolitan University staff were invited in July 2020 to participate in an antibody screening programme, 739 participants were tested during this study.

The cohort

Staff who worked from home, with immediate effect from 17th March 2020. Essential staff only returning to campus on a part time basis, where appropriate COVID-19 risk could be mitigated.

Screening for SARS-CoV-2 IgM and IgG antibodies

Volunteers were invited to participate in phase 1 of screening via the University staff portal and newsletter and booked an individual appointments at the facility using an online book- ing system (Outlook) where they were assigned a unique identifying code. A detailed participant information sheet was also provided through this medium.

All participants were asked to fill in a brief background and medical questionnaire prior to each test. This questionnaire included questions relating directly to COVID-19 exposure and perceived risk at both time points, as well as gathering general health information.

During the allotted 30min appointment, rapid testing was carried out using the FDA approved Confirm BioSciences ® (California) test against SARS-CoV-2, for both IgM and IgG antibodies using the manufacturer's guidelines. Briefly, finger prick blood (capillary) samples were collected from all participants and approximately 10µL of capillary blood was transferred into the test window. Buffer (provided with test) was added to the sample and the test was left to develop. Following 10 minutes the test window was read and interpreted as suggested by the manufacturer guidelines. Confirm Biosciences® report a specificity of 98% and sensitivity of 100% for this test when used in clinical samples (Specificity and sensitivity have been disputed by FDA; however, the test remains commercially available).

All those who tested positive for IgM and/or IgG antibodies and a matched number of individuals who tested negative were randomly selected to give a venous blood sample. Serum was isolated from these samples and sent to the Specialist Virology Centre, UHW to determine antibody titres using gold standard Abbott® and Euroimmun® antibody tests. These tests were undertaken entirely independently and blind to the screening result and to subject details.

Three months following the original antibody test, subjects who tested positive and those selected previously as negative controls were invited to be re-tested in phase 2 of screening. This test was a repeat of the original rapid antibody test using

capillary blood and the collection of a venous blood sample for analyses.

Ethics

This study was approved by the Cardiff Metropolitan University Ethics Committee (ref# sta-2860) and was conducted in line with the principles outlined in the Declaration of Helsinki. Standard operating procedures were approved and monitored throughout by University Health and Safety and the COVID-19 Response Group as part of the study risk assessment.

Statistical analysis

Test sensitivity and specificity was determined using a standard binary classification test, which determined matched negative and positive results across the 3 different antibody tests undertaken. Health questionnaire and participant data were logged online and later analysed using the University secure Qualtrics based system. Where two groups were compared an unpaired T-test was used to determine differences between groups. Significance is determined as $p<0.05$.

Results

Phase 1 Results

By LFT antibody test, 3.65% of the 739-population tested positive for COVID-19 antibodies (IgM and/or IgG).

Furthering this, 26 participants had been previously tested for COVID-19 virus, via PCR through the Public Health Wales COVID-19 testing units. Of these, 3 participants were tested virus positive, however only 1 of these participants showed positive for either IgM or IgG COVID-19 antibodies in our study.

Of those tested, 5% of the male population and 2.73% of the female population tested positive for COVID-19 antibodies. The average age of positive participants was 44.5 years vs. 42.91years for negative participants (cohort ages detailed below in table 1). Of those participants who tested positive for COVID-19 antibodies, 34.6% were aged 40 and under. The highest prevalence of COVID-19 antibodies was observed in males aged between 61 and 70 (6.67% prevalence).

The medical questionnaire was completed by all participants at both testing appointments. From the population data collected a disproportionate risk is highlighted in those with pre-existing health conditions. Participants who reported being asthmatic made up 33.3% of the positive antibody population, while 7.4% reported being diagnosed with hypertension. A minority of the positive cohort reported a further underlying condition, including type 1 diabetes or cardiovascular pathology.

Smoking did not associate with positive COVID-19 antibody tests, with 4.55% of the positive population indicating they smoke regularly. Smokers made up a comparable 5.5% of the antibody negative population.

Phase 2 Results

All participants whom tested antibody positive and a matched number of antibody negative controls were invited back for screening in phase 2 of the study. Of those invited, 79.69% returned.

Of those who originally tested negative 96.43% remained negative and 3.57% showed new antibody positivity. Of those whom originally tested positive 78.26% remained positive, however 21.74% showed a negative test result. Of those whom sera-converted from antibody positive to antibody negative 80% were female.

Comparison of antibody test methodologies

Blinded comparison between all positive antibody tests and randomly selected negative controls, of LFT and two independent antibody testing methods was undertaken. The prevalence data collected from LFT showed 92.85% agreement with the Euroimmun® (Figure 1a) and 78.57% agreement with the Abbott Diagnostic® test (Figure 1b).

Comparison with both Abbot and Euroimmun® antibody tests showed our test to have a specificity of 95% and a sensitivity of 89%. It is important to note that both these tests detect only IgG presence. LFT also detects IgM and given only IgM was detected in an additional 7% of participants, this results in an overall sensitivity of 96%.

Gender differences in antibody titre

Antibody titres between male and female positive participants were compared for both the Euroimmun® (Figure 2A) and Abbott® (Figure 2B) antibody tests. The Euroimmun® test shows no significant difference in positive antibody titres between male and female participants. However, in the Abbott® test males had significantly ($p=0.0024$) higher antibody titres than antibody positive females.

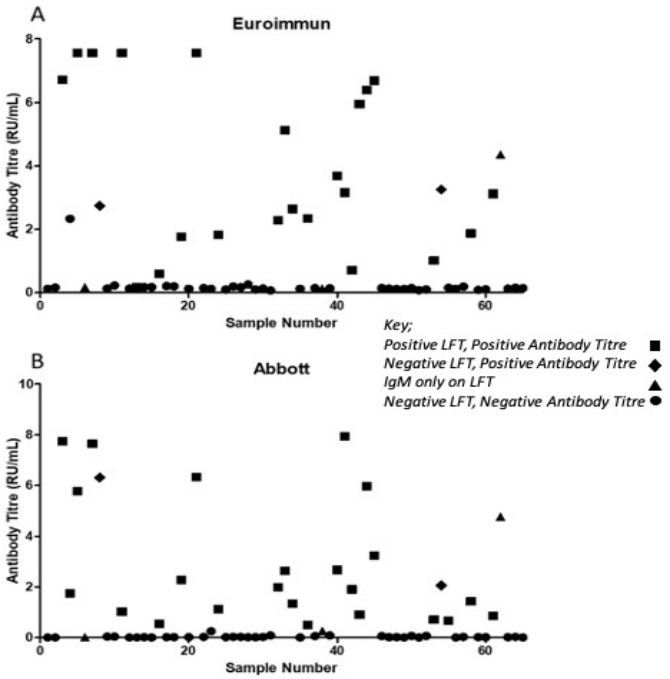

Figure 1: Comparison between LFT results and **(A)** Euroimmun® antibody titre and **(B)** Abbott Diagnostic® antibody titre.

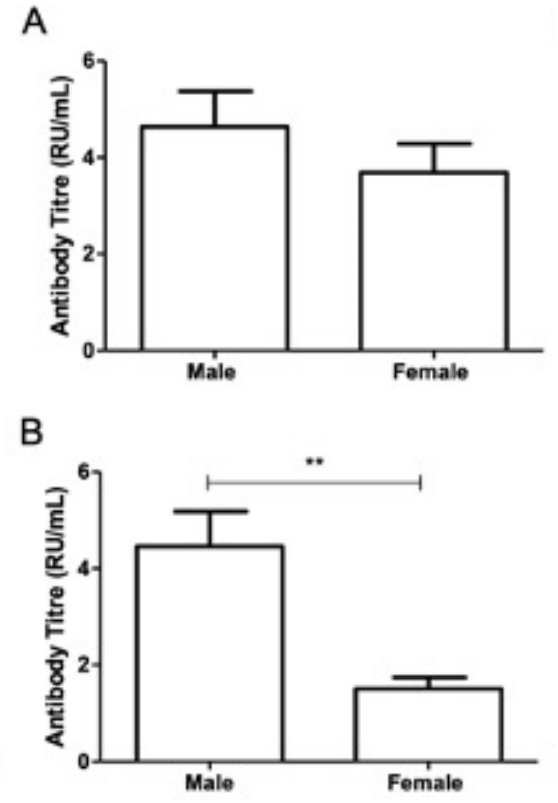

Figure 2: Antibody Titres in positive males and females**.** Comparison of antibody titres in **(A)** Euroimmun® antibody test and **(B)** Abbott ® antibody test. Each compare blood antibody titres in both male (N=13) and female (N=10) participants from phase 1 of the study. **=p<0.01.

Table 1: Cohort Age Data.

	Total Number of Participants	Total Number of Females	Total Number of Males
Total Number Tested	739	439	300
Positive IgM +/- IgG	28	13	15
Average Age	43.45 Years	43.3 Years	43.63 Years
Average Positive Age	44.5 Years	43.21 Years	45.79 Years
Average Negative Age	42.91 Years	42.3 Years	43.52 Years

Discussion

This study brings important data to the ever-progressing field of COVID-19 antibody prevalence and is a first of its kind study to demonstrate the change in antibody prevalence in a single cohort of asymptomatic participants over 6 months in Wales. We confirm the usefulness of rapid screening using LFT in comparison to standard methodology and apply these to identify a potential difference in the longevity of antibodies between males and females.

The reason why male and female antibody prevalence differs in this asymptomatic cohort (5% v 2%, respectively) is currently unknown, but could be due to an impaired immune response in post-menopausal women, linked to the loss of sex hormones [11]. The females who sera-converted in this study were on average 10 years older than those females whom maintained antibody positivity (mean age 54.5Years v. 45.5Years respectively). A significant difference is also seen in the antibody titres measured in male versus female antibody positive participants, with females exhibiting significantly lower levels. This may go some way in explaining why 80% of those who became antibody negative in phase 2 of this study were females. Similar gender differences have been observed with the effectiveness of both seasonal and pandemic influenza vaccinations and are linked with, cytokine, antibody and memory immune responses [12].

The fact that gender differences exist in both morbidity and mortality associated with COVID-19 has recently been identified [18]. A recent, large-scale meta-analysis has determined that although there is no difference in the proportion of males and females being infected with COVID-19, males are three times more likely than females to require ICU treatment and have higher mortality following infection [18]. This study also confirms males to have a higher antibody titre than females with a similar viral exposure, with females showing a higher T-cell response [18]. Further studies are required to determine why females having lower antibody titre in response to COVID-19 infection do not progress to have disease as severe as their male counterparts. Sex differences in both innate and adaptive immunity go some way in explaining this potential disparity, where females have a higher number of CD4+ [19] and CD8+ [20] T-cells, offering a further level of immune protection. Further work should focus on how antibody lifetime dictates susceptibility to ongoing effects of COVID-19, for example, long COVID, or indeed future infection with COVID-19.

The measured antibody prevalence of 3.65% is lower than the suggested national average (4-6%), as suggested by the UK REACT study, that showed antibody prevalence in July across the UK was 6%, decreasing to 4.8% in August and 4.4% in September [5]. Prevalence is also lower than that suggested by UK Biobank (4.7%) [4] based on a similar sized Welsh cohort, which also highlighted greater prevalence in areas of high population density (5.8% in Cardiff, 10% London) ranging from 2.8% in rural locations. A lower prevalence of COVID-19 antibodies was potentially seen in our cohort of professional staff as the vast majority were able and advised to work from home during the period March through September 2020, with a continuation where possible (applying a blended mix of 30% face-to-face teaching and 70% online learning) throughout October-December, suggesting a potential lower viral exposure.

The results presented in this study agree with numerous studies which suggest that age is a key determinant in COVID-19 infection. However, the results potentially disagree with those suggesting that lower infection rates are seen in older participants [4], since 65.4% of the antibody positive participants in our study were aged 41 and above, with highest prevalence occurring in males aged 61-70 years. Thus, it may be suggested on the basis of antibody prevalence, of the working population, older employees are potentially more at risk of COVID-19 than their younger colleagues.

This study reports an LFT method that has both high sensitivity (96%) and specificity (95%) used to confirm presence of COVID-19 antibodies. The practical benefits of using a sensitive and specific LFT for antibodies could aid our understanding of antibody prevalence in large populations, as well as the potential in tracking antibody response over time following COVID-19 vaccination. None of the antibody positive participants in this study suffered with COVID-19 related symptoms during the follow up time period, which might be taken to imply a level of protection against re-infection.

LFT has an important role to play in virus and antibody detection. Its benefits include being; safe, fast, cheap and high-throughput, from a single finger prick blood sample, that avoids the need for laboratory analysis, and in terms of practicality for population screening outweigh claims of LFT having low sensitivity and specificity, as confirmed here. LFT kits can be widely applied and require minimal training, however, in order to yield wide scale population data, it is important that standardization across the multitude of suppliers is introduced. A similar LFT based approach has been applied to screen local populations in areas of high incidence of COVID-19, where the LFT applied detects SARS-CoV-2 virus, and has informed the widescale screening of the public [16,17].

Critically, COVID-19 vaccination is now being rolled out across the UK, with vaccination of front-line health care staff and the elderly already underway. Our results confirm LFT offers the ability to monitor individual antibody production and thus the effectiveness of COVID-19 vaccination on a wide scale. This data could be collected over time to aid the understanding of COVID-19 antibody longevity and the effectiveness of antibody production in the prevention of COVID-19 infection.

Limitations

It is acknowledged we have not at any time tested the participants in this study for the presence of virus antigen and this could be considered a limitation of this study. We report however, on antibody prevalence in participants who either reported none or reported experiencing mild COVID-19 associated symptoms at any point in the previous 6 months. These participants were a-symptomatic at the time of testing for COVID-19 antibodies and did not report a CRP level (indicative of recent acute infection) outside of the normal range (data not shown).

We accept that this is a study of a relatively small and specific cohort and therefore may be classed as a limitation of this study. Importantly, this cohort equally represented both genders and covered a vast age range from 18 to 70 years old. Its specificity allows targeted results to be formed on this professional Welsh cohort.

Conclusions

High sensitivity and specificity LFT for identification of antibodies against COVID-19 performs extremely well when compared against standard methods, and confirms this could be rolled out at low cost and with minimal user training. Applying LFT we confirm an antibody prevalence in our asymptomatic population of 3.65%. Within 6 months, 79.69% of antibody positive participants retained antibodies, with females being 4 times more likely to lose antibodies against COVID-19.

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