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**Mood and Influenza Vaccination in Older Adults: A Randomised Controlled Trial**

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**Conflicts of interest**

All authors have no conflicts of interest to declare.

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## Abstract

### Objective

Positive mood on the day of vaccination has been associated with subsequent antibody responses to the influenza vaccine in older adults. The primary aim of this trial was to examine whether a brief intervention was able to enhance positive mood at the time of vaccination in a clinical context. Secondary aims included exploratory analyses of the effects of the intervention on non-specific and influenza-specific immunity.

### Methods

One hundred and three older adults (65-85 years) participated in a two-arm, parallel, single-blind, randomised controlled trial. Participants viewed either a 15-minute video package designed to induce positive mood or a matched neutral control video, immediately prior to receiving a standard dose quadrivalent influenza vaccination. State affect and secretory IgA levels were assessed immediately prior to, and following, the interventions. Antigen-specific IgG responses to the vaccination were assessed at 4 and 16-weeks post-vaccination.

### Results

The positive mood intervention resulted in significant improvements in state positive affect, compared with the neutral control. Secretory IgA levels significantly increased across both groups. Antigen-specific IgG responses to influenza vaccination were not statistically significantly different between groups, although point-estimates of effect size favoured participants who viewed the positive mood intervention for most strains at both 4 and 16-weeks post-vaccination.

### Conclusions

A 15-minute intervention can improve positive mood in older adults prior to vaccination. Future trials should examine whether enhancing mood at the time of vaccination could enhance the effectiveness of influenza vaccination on patients and benefit health services.

*Keywords: Positive affect, intervention, positive mood, vaccination, immunity, psychoneuroimmunology*

TRIAL REGISTRATION: ClinicalTrials.gov identifier: NCT03144518

The protection afforded by the annual seasonal influenza vaccination is considerably lower in older adults compared to younger adults (Goodwin, Viboud, & Simonsen, 2006), in part due to age-related declines in immunological competence (Reber et al., 2012). This leaves older adults at an increased risk of the most serious complications associated with influenza infection, with 90% of influenza-related deaths in industrialised countries occurring in those aged over 65 years (Thompson, Shay, & Weintraub, 2003). To date, advances to improve influenza vaccine responses in older adults have primarily focused on pharmacological solutions. These have met with some success with adjuvanted influenza vaccines, that contain an additional component that stimulates a heightened immune response, and high-dose vaccines, that include a four times greater amount of each influenza antigen, showing improved protection in older adults in recent years (DiazGranados et al., 2014; Domnich et al., 2017). However, even with the increasing adoption of these enhanced vaccine formulations, reduced vaccine efficacy in older adults remains problematic. For example, the adjuvanted trivalent (i.e., containing three strains) influenza vaccine adopted by many countries for the most recent influenza season (2018/19), was reported to have an estimated effectiveness of around 51% against hospitalisations for influenza/pneumonia in community dwelling older adults (Domnich et al., 2017). This indicates calls to research and develop novel approaches to improve vaccine responses in this population (e.g., Kelly & Valenciano, 2012; Lang, Govind, Mitchell, Siegrist, & Aspinall, 2011; Lang et al., 2012) remain highly relevant.

One alternative to enhancing vaccine responses in older adults are psychological and behavioural interventions. Psychological and behavioural factors have been shown to influence the dynamics of the immune system, in turn modifying responses to antigenic challenge (e.g., Calder, 2013; Pascoe, Fiatarone Singh, & Edwards, 2014; Pedersen, Zachariae, & Bovbjerg, 2009; Prather et al., 2012). To give some examples: chronically

stressed older adult spousal carers show blunted immune responses to influenza vaccination compared to age-matched non-carers (Kiecolt-Glaser, Glaser, Gravenstein, Malarkey, & Sheridan, 1996; Vedhara et al., 1999); a single night of sleep deprivation in young adults results in a nearly two-fold reduced antibody response to hepatitis A vaccination at 4 weeks post-vaccination compared to those who sleep normally (Lange, 2003); and salivary antibody secretions to an orally ingested antigen have been shown in longitudinal studies to be differentially influenced by both positive and negative life events (Stone, Cox, Valdimarsdottir, Jandorf, & Neale, 1987; Stone et al., 1994). More recently in a prospective cohort study examining the effects of multiple lifestyle factors and psychological well-being on the response to influenza vaccination in older adults (Ayling et al., 2018), we identified that positive affect, in particular on the day of vaccination, was associated with enhanced short and long-term antibody responses to the weakest immunogenic strain of the influenza vaccination.

The mechanisms by which positive affect influences immune function continue to be elucidated (for reviews of this topic see Dockray & Steptoe, 2010; Marsland, Pressman, & Cohen, 2007; Pressman, Jenkins, & Moskowitz, 2019). Indirect influences of positive affect via health behaviours have been shown to impact immune function (e.g., sleep, nutrition, physical activity). Similarly, direct effects have also been reported via the activation of neuroendocrine systems responsible for the release of hormones, such as cortisol and norepinephrine, which in turn interact with immune cells via receptors on their surface (Marques-Deak, Cizza, & Sternberg, 2005). Further, there are well established anatomical links between the central nervous and immune systems, including the direct innervation of lymphatic organs (Felten & Felten, 1991; Nance & Sanders, 2007) that can potentially be triggered by positive affect. As such there are multiple, biologically plausible mechanisms by

which a positive affect intervention could influence immunity, and in turn, antibody responses to vaccination.

In view of previous observational evidence that positive mood on the day of vaccination is associated with enhanced antibody responses; and the presence of plausible biological pathways to explain this effect, we designed a study to explore whether we could enhance positive mood in older people immediately prior to vaccination. The study was a randomised controlled trial in which participants were randomised to view either a novel positive mood intervention on a digital platform (15 minutes long) or neutral control material also 15 minutes in duration and delivered on the same digital platform. Our primary aim was, therefore, to examine whether our positive mood intervention resulted in greater positive mood compared with the neutral control. The secondary aims, were to: explore the effect on an immediate immune parameter (secretory IgA) previously shown to be sensitive to positive mood inductions (e.g., Pawlow & Jones, 2005; Yamamoto & Nagata, 2011) and vaccine-specific IgG responses, as a surrogate of vaccine effectiveness. We also examined the moderating effects of perceived health status and psychological traits on mood and immune responses to the intervention; and the feasibility of a future definitive trial by examining participant recruitment levels, attrition, and numbers receiving the intervention as intended.

## Method

### Study Design

The study was a two-arm, parallel, single-blind, randomised controlled trial conducted between August 2017 and May 2018. Research governance and ethical approval for this study was given by the Health Research Authority and East Midlands - Nottingham Research Ethics Committee 1 (17/EM/0198) prior to study commencement. The study was pre-registered at clinicaltrials.gov (NCT03144518).

## Participants

One hundred and three older adults (65-85 years) eligible to receive the 2017-18 influenza vaccination participated in the study. Participants were identified through two primary care practices in Nottingham, UK. Given the influence of prior vaccination history on subsequent immune responses to vaccination (Sasaki et al., 2008), recruitment was limited to those who had received the influenza vaccination the previous year (2016/17). The purpose of this was to minimise between-person differences in previous exposure to influenza and influenza vaccination and the concomitant effects on antibody levels prior to vaccination. Alternative approaches including statistically controlling for vaccine history or recruiting only participants who had not previously received an influenza vaccination were rejected due to concerns with poor record keeping and reduced generalisability respectively. Exclusion criteria were kept minimal to maximise generalisability. They included: deemed by health care provider to be too physically frail to participate; diagnosed with a cognitive condition (e.g., dementia) that would make participation difficult; to have insufficient command of the English language; being sufficiently impaired of hearing or vision that exposure to the intervention or neutral video content as intended would be compromised; or having a contraindication for influenza vaccination or venepuncture.

## Randomisation

Randomisation to condition was done using a computer-generated block randomisation sequence (block size = 10) on a 1:1 allocation ratio, initiated by the lead author (KA). This randomly generated sequence was matched to participant ID numbers (assigned in order of response to invitation letter) in such a manner that touchscreen computer tablets were programmed to play either the positive mood or neutral control content, without the researchers being aware of participant assignment.

## Procedure

Eligible patients at each primary care practice were sent an invitation letter detailing the study and providing researcher contact details. Participants were informed the purpose of the study was to explore the effects of mood on influenza vaccination and that they would be asked to watch a short video. They were not told that there were positive mood and neutral control conditions. Interested individuals contacted the research team via email, telephone, or by returning a reply slip provided with a freepost envelope. After discussing the study with the researchers, those who agreed to participate were sent a detailed information sheet, consent form, and baseline questionnaire to complete and return via post. This questionnaire included sections on demographic information, perceived health status, and trait psychological characteristics related to affective experience.

Participants then attended a session at their local primary care practice (maximum of six participants per session) where they had a pre-vaccination blood sample taken (8ml), height and weight measured, and provided a pre-intervention saliva sample for the measurement of secretory IgA. Participants were then given a demonstration of how to use a touchscreen computer tablet device (Model: ASUS-T101HA) to complete the state affect measures, with a small minority opting to complete measures using printed questionnaires ( $n=3$ , 2.9%). Participants then completed pre-intervention measures (approximately 5 minutes) and viewed either the positive mood intervention video or neutral control video on an individual tablet device wearing over-ear headphones, followed by completion of the post-intervention measures. For those completing measures on the tablet (presented using OpenSesame software: Mathot, Schreij, & Theeuwes, 2012) - the order of scale presentation (and any items within those scales) was randomised to minimise order effects. Participants then provided a further saliva sample and had a standard dose of the quadrivalent 2017/18 seasonal influenza vaccine administered. The vaccine was administered by a practice nurse or

health care assistant within five minutes of completing the intervention. Participants returned for follow-up blood samples at four and 16-weeks post-vaccination. Health-care utilization attributable to influenza-like symptoms was assessed by the lead author (KA) who consulted medical records at six months post-vaccination noting any consultations during the prior six months where influenza-like symptoms were assigned read-codes or noted in free-text entries (e.g., runny nose, sore throat, fever), antibiotic prescriptions not clearly attributable to another cause (e.g., urinary tract infection), and where additional investigations related to influenza-like symptoms were ordered (e.g., chest X-ray). Participants received £20 inconvenience payments for taking part in the study. Materials and stimuli used in the study can be accessed at osf.io/u7h36.<sup>1</sup>

### **Positive Mood Intervention Condition**

Participants in the positive mood condition viewed a video lasting 15 minutes and 30 seconds specifically developed to increase state affect in older adults. Most previous brief positive affect interventions have been developed for use in young adults and without a clinical context in mind. The development of the intervention is the subject of a related manuscript (Ayling et al., in preparation). In brief, the intervention was iteratively developed following a systematic review of brief interventions that induced positive mood and measured immunity, qualitative focus groups and interviews with older adults and health care professionals, and co-development alongside patient and public involvement partners. These led to some key guiding principles that the intervention should be presented individually, made up of high arousal stimuli in multiple forms (e.g., uplifting music, images, ‘classic’ comedy) to appeal to different tastes, and be around 15 minutes in duration due to the constraints of a primary care clinical context. Thus, the resulting intervention was a

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<sup>1</sup> Please note that while UK copyright laws permitted the limited use of copyright protected works used in the interventions for non-commercial research purposes in the UK at the time this research was conducted (see <https://www.gov.uk/guidance/exceptions-to-copyright>), copyright restrictions may apply in other countries or as a result of subsequent legislation changes in the UK.

combination of these elements. A description of the specific intervention stimuli and the intervention itself can be viewed at osf.io/u7h36. Prior piloting of the intervention in older adults outside of a clinical context indicated that the intervention was successful in inducing significant positive affect increases assessed on a visual analogue scale of happiness (Kontou, Thomas, & Lincoln, 2012), and a pictorial scale of positive affect (see below), but not the positive and negative affect schedule (Watson, Clark, & Tellegen, 1988).

### **Neutral Control Video**

Participants in the control condition viewed a video designed to match key features of the positive mood intervention video (e.g., length, format), but with neutral emotional content. For example, videos included documentary and lecture clips, music judged to be non-emotive, and images with neutral emotional valence from the Nencki Affective Picture System (Marchewka, Zurawski, Jednoróg, & Grabowska, 2014).

### **Measures**

**Baseline/trait measures.** Perceived health status was measured using the 12 item Health Status Questionnaire 2.0 (Barry, Kaiser, & Atwood, 2007). Participants indicated the extent to which they experienced a variety of health-related difficulties (e.g., “How much bodily pain have you had during the past 4 weeks?”) on Likert-type scales. Items covered a broad range of health-relevant domains including physical functioning, social functioning, bodily pain, and mental health. Responses were weighted in accordance with published guidelines and summed to calculate a total perceived health status score ( $\alpha = .91$ ), with higher scores indicating greater perceived health.

Trait positive and negative affect were measured using the Positive and Negative Affect Schedule (Watson et al., 1988). Participants were presented with 10 positive (e.g., excited) and 10 negative (e.g., ashamed) emotion adjectives, which they were asked to rate the extent to which they felt that way “in general” on a five-point scale (“very slightly or not

at all” – “extremely)”. Positive ( $\alpha = .92$ ) and negative affect subscales ( $\alpha = .92$ ) were created by summing the scores of positive and negative adjectives respectively, with higher scores indicating greater trait positive and negative affect respectively.

Trait optimism was measured using the Revised Life Orientation Test (Scheier, Carver, & Bridges, 1994). Participants were asked to indicate the extent to which they agreed with 10 statements about themselves (e.g. “In uncertain times, I usually expect the best”) on a five-point scale ranging from “strongly disagree” to “strongly agree”. Four items were filler questions, with the remaining six used to compute a trait optimism score ( $\alpha = .73$ ) with higher scores indicating with greater optimism.

Trait emotional reactivity was measured using the 21-item Emotional Reactivity Scale (Nock, Wedig, Holmberg, & Hooley, 2008). Participants were asked to indicate the extent to which they felt particular statements reflect themselves (e.g., “I experience emotions very strongly”) on a five-point scale ranging from “not at all like me” to “completely like me”. Items scores were then summed ( $\alpha = .96$ ), with greater scores indicating greater trait emotional reactivity.

**State affect measures.** Prior to, and immediately following, viewing either the positive mood or neutral control video all participants completed the Affective Slider (Betella & Verschure, 2016), the state version of the Positive and Negative Affect Schedule (Watson et al., 1988), and an internally developed pictorial measure of positive affect tailored for older adults (described below). The primary outcome measure was the Affective Slider, as prior piloting of the intervention in community samples raised concerns regarding sensitivity of the Positive and Negative Affect Schedule to acute changes in mood, and the pictorial measure had not yet gone through robust reliability testing.

**Affective slider.** Participants were asked to place a mark representing “how you feel right now, that is, at the present moment”, on two horizontal sliding visual analogue scales

representing emotional valence and arousal. Each scale was presented with corresponding cartoon faces depicting high and low levels of pleasure and arousal respectively. Scores range from 0 to 100 with higher scores indicating greater pleasure and arousal (hereafter referred to as slider-valence and slider-arousal scores). The Affective Slider has previously been shown to compare favourably against the Self-Assessment Manikin and be responsive to affective stimuli (Betella & Verschure, 2016).

***Positive and negative affect schedule (PANAS).*** The state version of this scale (Watson et al., 1988) consists of 20 emotion adjectives for which participants indicated the extent to which they felt that way “right now, that is, at the present moment” - on a five-point scale. Positive (mean  $\alpha = .89$ ) and negative affect subscales (mean  $\alpha = .89$ ) were created by summing the scores of positive and negative adjectives respectively.

***Pictorial scale of positive affect.*** Participants completed a single-item photo-based measure of positive affect tailored for older adults, the development of which is the subject of a different manuscript (Ayling et al., in preparation). Participants were presented with six groups of images (including older adult faces) depicting varying degrees of positive affect, ranging from neutral to extremely happy. They were then asked to select the group of images which best reflected “how you feel right now, that is, at the present moment”. Piloting in community samples showed this scale was sensitive to mood induction, and significantly positively correlated with the neutral-happy dimension of the revised Visual Analogue Mood Scale (Kontou et al., 2012), and the state version of the PANAS positive affect scale.

### **Immune Measures**

**Antigen specific IgG.** Venous blood samples (8 ml) were obtained for the measurement of IgG antibody levels at baseline, 4 weeks post-vaccination, and 16 weeks post-vaccination. The rationale for the timings of blood samples was that IgG antibody levels are at their peak approximately 4 weeks post-vaccination (Gross et al., 1996), whereas 16

weeks post-vaccination coincides with the anticipated peak of influenza incidence in the UK (i.e., January-March) at which point sustained high levels of antibody may be of particular clinical benefit. After clotting at room temperature, samples were centrifuged at 2000g for 10 minutes after which sera were separated and stored at -20°C until analysis. Influenza IgG antibodies for each of the strains contained in the vaccine were measured via enzyme-linked immunoassay (ELISA). The following description outlines the protocol used, with aspiration and washes with three cycles of 80 µl 0.05% Tween-20 solution in phosphate buffered saline (PBS) occurring between each step, with all incubations at room temperature unless otherwise stated. All samples were analysed in duplicate (samples mean intra-assay CV = 3.97%) with dilutions and pipetting into plates were performed by using a computerised pipetting system (Precision XS, BioTek) for accuracy.

Antigens (A/Hong-Kong/4801/2014; A/Michigan/45/2015; B/Brisbane/60/2008; B/Phucket/3073/2013; National Institute of Biological Standards and Control) were diluted to 0.5 µgHA/ml, 1 µgHA/ml, 0.25 µgHA/ml, and 0.25 µgHA/ml respectively in carbonate-bicarbonate buffer with 20 µl added to 384-well plates (NUNC MaxiSorp, Thermo Fisher Scientific). Alongside this, 15 two-fold serial human IgG dilutions (top dilution 2 µg/ml; Merek) were added in duplicate (20 µl) to each plate to form a calibration curve and left at 4°C overnight. Wells were then blocked with 40 µl of 3% bovine serum albumin in PBS for 1 hour. Sera diluted at 1:4000 in PBS was added in duplicate (20 µl) for each antigen for two hours. PBS was added to the calibration curve for the same period. Following this, 20 µl of biotinylated anti-human IgG (vector labs) diluted to 1:320000 in PBS was added for a further 2 hours. Streptavidin-HRP (R&D systems) diluted at 1:40 in PBS was then added to wells for 15 minutes. Finally, 20 µl of tetramethylbenzidine substrate solution (Sigma-Aldrich) was added to each well for 10 min, before the reaction was stopped with 20 µl of 1N sulphuric acid. Plates were then scanned at 450 nm on a GloMax Explorer instrument (Promega).

Signals from samples were interpolated from the human IgG calibration curve on each plate, with a set of 40 repeated samples processed on each plate used to normalise assays run across different days. Values were then multiplied by the serum dilution score (i.e., 4000) for ease of data presentation.

**Secretory IgA.** Unstimulated passive drool saliva samples were obtained immediately prior to, and following, viewing the positive mood or neutral control video. Participants were asked to allow saliva to accumulate in their mouth for two minutes, passing saliva into polypropylene vials through a SalivaBio collection aid (Salimetrics/Stratech). All vials were weighed before and after saliva collection to determine salivary flow rate. Samples were stored at -20°C until analysis. Secretory IgA levels were determined using a commercial indirect ELISA kit, according to manufacturer instructions (see Salimetrics, 2017). Secretory IgA secretion rate ( $\mu\text{g}/\text{min}$ ) was calculated by multiplying secretory IgA concentration from the assay by salivary flow rate, divided by the total collection time.

### Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (version 25) and GraphPad Prism (version 7). The primary outcome was between group differences in state affect following the intervention or neutral control video. *A priori* sample size calculations indicated a sample of 102 participants would be needed to detect a medium-sized ( $d=0.5$ ) between group effect in state affect scores in a one-tailed independent samples t-test with 80% power. A recent meta-analysis we conducted of brief-mood enhancing interventions and immunity (Ayling et al., under review) suggested that successful brief (i.e., single session) interventions tend to have an average effect size on mood of around  $d=0.6$ , although there was significant heterogeneity. However, only one of the trials in that review included older adults and it is not yet established how large a mood effect would have to be to be ‘immune-relevant’. Given this, we decided *a priori* that a medium sized effect on mood would be

comparable to other successful mood manipulations in younger cohorts and thus powered the trial accordingly. However, given our focus on between group differences we conducted a series of two-way analyses of variance comparing change in state affect scores across groups. Normality of distributions were assessed via the examination of histograms, with log<sub>2</sub> or reciprocal transformations used to improve normality where possible if significant skew was evident. Paired sample t-tests, or non-parametric equivalents were used to explore within-group effects. The potential influence of trait psychological characteristics on state affect responses was also explored. This involved a series of multiple regression analyses entering trait measures individually to predict post-intervention state affect scores controlling for pre-intervention scores. Those trait measures that significantly predicted post-intervention state affect scores were entered simultaneously in a further model to test whether they were independent predictors.

This trial was not *a priori* powered to detect significant between group differences in immunological outcomes but to determine if there was early evidence of an effect of the intervention on immunity and to explore the feasibility of a subsequent, appropriately powered trial. As such, results for immunological outcomes are primarily presented descriptively, with inferential tests conducted for the purpose of describing effect sizes. For secretory IgA outcomes we conducted a two-way analysis of variance comparing change from pre- to post-intervention between groups. For antigen specific IgG levels we conducted a series of one-way analyses of covariance comparing between group post-intervention IgG levels, in order to better control for pre-intervention differences. Given prior findings that trait psychological characteristics and health status can influence immunological responses to vaccination (Gross, Quinnan, Weksler, Setia, & Douglas, 1989; Marsland, Cohen, Rabin, & Manuck, 2006), we explored this across the entire cohort. To do this, a series of multiple regression analyses predicting post-intervention antigen-specific IgG were conducted

controlling for pre-intervention IgG levels and condition in step one, with trait psychological characteristics and health status entered individually in step two. Where multiple significant predictors were identified they were entered simultaneously in a further model to test whether they were independent predictors.

As levels of missing outcome data were low (see supplementary appendix) and found to be missing completely at random (Little's MCAR test:  $\chi^2(237) = 236.12, p = .504$ ), we performed intention-to-treat analyses with listwise deletion for analyses where follow-up data were missing. We also performed per-protocol analyses excluding three participants (two from neutral control group, one from positive mood group) who did not complete study activities as intended (one was observed putting the tablet down and not watching the presented stimuli, one was observed watching another nearby participant's tablet screen, and one was delayed in receiving their vaccine after completing tablet activities). Nearly all per-protocol analyses did not result in any differing interpretations of the results presented below, thus only those which potentially impact on findings are presented here.

## Results

### Participant Recruitment & Randomisation

Figure 1 shows the flow of participants through the study. Of the 1131 patients invited to participate, 106 (9.4%) consented. Of these 103 (97.2%) attended the appointment to receive their vaccination and were randomised to view the intervention or neutral control video. Retention throughout the study was high, with 102 (96.2%) participants attending follow-ups at four weeks post-vaccination, and 98 attending follow-ups at 16 weeks post-vaccination (92.5%). Table 1 shows the baseline characteristics of participants in each group. Pre-intervention there were no significant differences between groups on any demographic, psychological, or immunological factors.

### Psychological Outcomes

**State affect outcomes.** We performed two-way mixed analyses of variance to compare the effects of the positive mood intervention and neutral control videos on state affect. Pre- and post-intervention scores on state affect measures can be seen in Table 2. Participants in the positive mood group had significantly greater improvements from pre- to post-intervention for slider valence [ $F(1,98) = 6.97, p = .010$ , partial  $\eta^2 = .066$ , 90% CI (.009, .157)], and pictorial positive affect scores [ $F(1,96) = 8.83, p = .004$ , partial  $\eta^2 = .084$ , 90% CI (.017, .181)] as indicated by significant time by group interactions. There were no statistically significant time by group interactions for slider-arousal [ $F(1,98) = 3.36, p = .070$ , partial  $\eta^2 = .033$ , 90% CI (.000, .108)] or PANAS scores [positive affect:  $F(1,99) = 0.19, p = .668$ , partial  $\eta^2 = .002$ , 90% CI (.000, .039); negative affect:  $F(1,101) = 0.22, p = .642$ , partial  $\eta^2 = .002$ , 90% CI (.000, .040)].

Paired Wilcoxon signed rank tests examining state affect in the groups individually showed that following the intervention the positive mood group significantly increased in slider-valence ( $Z = -4.21, p < .001, r = .60$ ), slider-arousal ( $Z = -2.92, p = .003, r = .42$ ), and pictorial positive affect scores ( $Z = -3.70, p < .001, r = .53$ ), significantly decreased in PANAS negative affect scores ( $Z = -3.95, p < .001, r = -.51$ ), but had no statistically significant change in PANAS positive affect scores ( $Z = -1.66, p = .098, r = .23$ ). In contrast, there were no significant changes in the neutral control group following viewing the video (Slider-Valence:  $Z = -.24, p = .810, r = .03$ ; Slider-Arousal:  $Z = -.11, p = .912, r = -.02$ ; pictorial positive affect scores:  $Z = -.13, p = .895, r = -.02$ ; PANAS negative affect:  $Z = -1.93, p = .053, r = -.27$ ; PANAS positive affect:  $Z = -.64, p = .524, r = .09$ ). In per protocol analyses, the decrease in PANAS negative affect scores in the neutral control group became statistically significant ( $Z = -1.99, p = .046$ ).

**Influence of trait psychological characteristics on state affect outcomes.** Multiple regression analyses were conducted to examine the influence of trait psychological

characteristics on state affect responses. Within the positive mood group, higher slider-valence and slider-arousal scores following the intervention (controlling for pre-intervention scores) were significantly predicted by lower trait negative affect (slider-valence:  $\beta = -.312, p = .003$ . Model:  $F(2, 44) = 40.37, p < .001, R^2 = .631$ ; slider-arousal:  $\beta = -.348, p = .003$ . Model:  $F(2, 44) = 43.68, p < .001, R^2 = .665$ ) and trait emotional reactivity (slider-valence:  $\beta = -.274, p = .008$ . Model:  $F(2, 43) = 34.12, p < .001, R^2 = .613$ ; slider-arousal:  $\beta = -.247, p = .027$ . Model:  $F(2, 43) = 34.14, p < .001, R^2 = .614$ ) in separate regression models. Entering trait negative affect and emotional reactivity into regression models simultaneously indicated neither were independent significant predictors above pre-intervention scores<sup>2</sup>. Higher pictorial positive affect scores following the intervention were significantly predicted by lower trait negative affect ( $\beta = -.438, p = .001$ . Model:  $F(2, 43) = 10.92, p < .001, R^2 = .337$ ) and higher trait optimism ( $\beta = .305, p = .033$ . Model:  $F(2, 44) = 5.70, p = .006, R^2 = .206$ ) when considered separately. However, when entered into the model simultaneously only trait negative affect was an independent significant predictor ( $\beta = -.407, p = .008$ . Model:  $F(3, 40) = 7.61, p < .001, R^2 = .316$ ). No trait psychological characteristics significantly predicted state PANAS outcomes in the positive mood intervention group following the intervention. In the neutral control group, higher slider-arousal scores following the intervention were predicted by greater trait positive affect ( $\beta = .362, p = .016$ . Model:  $F(2, 42) = 6.48, p = .004, R^2 = .236$ ) and higher PANAS negative affect scores following the intervention were predicted by lower trait positive affect scores ( $\beta = -.357, p = .035$ . Model:  $F(2, 42) = 4.22, p = .021, R^2 = .17$ ).

## **Immunological Outcomes**

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<sup>2</sup> In per protocol analysis, for slider-arousal scores only trait negative affect was an independent significant predictor above pre-intervention scores ( $\beta = -.27, p = .048$ . Model:  $F(3, 40) = 28.41, p < .001, R^2 = .681$ )

**Secretory IgA.** Only 81 participants (37 neutral control group, 44 positive mood group) produced sufficient saliva for analysis at both pre- and post-intervention. Pre- and post-intervention s-IgA levels can be seen in Table 2. We conducted two-way mixed analyses of variance, to compare the effects of the positive mood intervention and neutral control videos on s-IgA secretion rates. There was no significant time by group interaction in post-intervention secretory IgA rates [ $F(1, 79) = .00, p = .996$ , partial  $\eta^2 < .001$ , 90% CI (.000, .000)]. There was however a significant main effect of time, such that secretory IgA increased over time across groups [ $F(1, 79) = 20.64, p < .001$ , partial  $\eta^2 = .207$ , 90% CI (.087, .327)]. Paired sample t-tests examining groups individually revealed secretory IgA rates significantly increased from pre-video to post-video samples in both positive mood intervention [ $t(43) = -3.36, p = .002, d = .51$ ] and neutral control groups [ $t(36) = -3.08, p = .004, d = .51$ ].

**Influenza-specific IgG.** As expected, IgG antibody levels for all strains significantly increased following vaccination [B/Brisbane:  $F(2, 192) = 27.78, p < .001$ , partial  $\eta^2 = .22$ , 90% CI (.139, .300); B/Phucket:  $F(1.85, 177.65) = 41.82, p < .001$ , partial  $\eta^2 = .30$ , 90% CI (.209, .382); A/Hong-Kong:  $F(2, 192) = 16.71, p < .001$ , partial  $\eta^2 = .15$ , 90% CI (.074, .219); A/Michigan:  $F(1.74, 165.68) = 36.90, p < .001$ , partial  $\eta^2 = .28$ , 90% CI (.183, .361)]. Pairwise comparisons (Bonferroni-adjusted) revealed that for both B strains, IgG levels significantly increased from baseline to four weeks post-vaccination (B/Brisbane:  $M_{\text{diff(log)}} = 0.24, p < .001$ ; B/Phucket:  $M_{\text{diff(log)}} = 0.48, p < .001$ ) and from four to 16 weeks post-vaccination (B/Brisbane:  $M_{\text{diff(log)}} = 0.14, p = .016$ ; B/Phucket:  $M_{\text{diff(log)}} = 0.24, p = .014$ ). For both A strains, IgG levels were significantly higher from baseline at both 4 and 16 weeks post-vaccination (A/Hong-Kong: four weeks -  $M_{\text{diff(log)}} = 0.34, p < .001$ ; 16 weeks -  $M_{\text{diff(log)}} = 0.24, p < .001$ ; A/Michigan: four weeks -  $M_{\text{diff(log)}} = 0.40, p < .001$ ; 16 weeks -  $M_{\text{diff(log)}} = 0.24, p < .001$ ).

$0.33, p < .001$ ), but did not significantly differ between four and 16 weeks post-vaccination (A/Hong-Kong:  $M_{\text{diff(log)}} = -0.10, p = .267$ ; A/Michigan:  $M_{\text{diff(log)}} = -0.08, p = .250$ ).

One way analyses of covariance showed that after adjusting for baseline antibody levels, mean post-vaccination antibody levels were not statistically significantly different between groups [A/Hong-Kong: four weeks -  $F(1, 99) = 0.61, p = .438$ , partial  $\eta^2 = .006$ , 90% CI (.000, .055); 16 weeks -  $F(1, 95) = 0.43, p = .516$ , partial  $\eta^2 = .004$ , 90% CI (.000, .051); A/Michigan: four weeks -  $F(1, 99) = 0.18, p = .671$ , partial  $\eta^2 = .002$ , 90% CI (.000, .038); 16 weeks -  $F(1, 94) < 0.01, p = .980$ , partial  $\eta^2 < .001$ , 90% CI (.000, .000); B/Brisbane: four weeks -  $F(1, 99) = 0.69, p = .409$ , partial  $\eta^2 = .007$ , 90% CI (.000, .057); 16 weeks -  $F(1, 95) = 0.79, p = .377$ , partial  $\eta^2 = .008$ , 90% CI (.000, .062); B/Phuket: four weeks -  $F(1, 99) = 0.02, p = .892$ , partial  $\eta^2 < .001$ , 90% CI (.000, .008); 16 weeks -  $F(1, 95) = 0.64, p = .426$ , partial  $\eta^2 = .007$ , 90% CI (.000, .058)] although point-estimates for all effect sizes were in the predicted direction, favouring the positive mood intervention over neutral control (see Figure 2). The only exception to this was in per protocol analyses, for A/Michigan IgG levels at 16 weeks post-vaccination.

**Influence of trait measures on IgG outcomes.** To examine the role of trait psychological characteristics and health status on IgG responses to vaccination, irrespective of group assignment, a series of linear regression models were performed. These showed trait positive affect was a significant independent predictor above pre-intervention IgG levels and condition of B/Phuket IgG levels at 16 weeks post-vaccination [ $\beta = .172, p = .045$ . Model:  $F(3, 86) = 18.30, p < .001, R^2 = .390, \Delta R^2 = .029, \Delta F(1, 86) = 4.15, p = .045$ ]. Trait negative affect significantly predicted the B/Phuket IgG levels at 4 weeks post-vaccination [ $\beta = .148, p = .037$ . Model:  $F(3, 87) = 39.00, p < .001, R^2 = .559, \Delta R^2 = .022, \Delta F(1, 87) = 4.48, p = .037$ ]. Both trait positive affect and optimism were predictive of B/Brisbane IgG levels at 16 weeks post-vaccination [trait positive affect:  $\beta = .147, p = .033$ . Model:  $F(3, 86) = 44.71, p < .001$ ,

$R^2 = .596$ ,  $\Delta R^2 = .021$ ,  $\Delta F(1, 86) = 4.71$ ,  $p = .033$ ; trait optimism:  $\beta = .132$ ,  $p = .049$ . Model:  $F(3, 87) = 47.51$ ,  $p < .001$ ,  $R^2 = .621$ ,  $\Delta R^2 = .017$ ,  $\Delta F(1, 87) = 3.99$ ,  $p = .049$ ] when considered individually, but when entered simultaneously neither were significant independent predictors. In per-protocol analyses, these trait predictors of B/Brisbane IgG at 16 weeks post-vaccination were not statistically significant (trait positive affect:  $\beta = .133$ ,  $p = .055$ . Model:  $F(3, 84) = 44.19$ ,  $p < .001$ ,  $R^2 = .612$ ,  $\Delta R^2 = .018$ ,  $\Delta F(1, 84) = 3.80$ ,  $p = .055$ ; trait optimism:  $\beta = .115$ ,  $p = .089$ . Model:  $F(3, 85) = 46.95$ ,  $p < .001$ ,  $R^2 = .624$ ,  $\Delta R^2 = .013$ ,  $\Delta F(1, 85) = 2.95$ ,  $p = .089$ ).

### Feasibility Measures

As shown in Figure 1, participant uptake was 9.4%. Other data on feasibility captured during the trial also suggested that most trial activities were well tolerated by participants. The only exception to this was the collection of saliva samples, which participants reported difficulties with and resulted in 21% of participants failing to produce samples. There were only three noted protocol violations and high levels of participant retention. Healthcare usage attributable to influenza-like symptoms during the six months were similar between groups (see supplementary appendix).

### Discussion

The primary aim of this trial was to examine whether a brief intervention was able to enhance positive mood at the time of vaccination in a clinical context. Secondary aims included exploratory analyses of the effects of the intervention on non-specific and influenza-specific immunity. Below, we discuss our findings in relation to each of these outcomes, as well as exploring the feasibility of a future larger trial.

### Intervention Effects on Mood

With regard to mood outcomes, we observed that in a clinical setting the positive mood intervention resulted in positive affect improvements, compared with a neutral control

intervention. The increase in positive affect was seen across all our state affect measures apart from the PANAS, for which negative affect scores decreased in both groups (though not significantly in the neutral control arm) and positive affect scores showed no change in either group. The reasons for the divergent effects the PANAS positive affect subscale are unclear. One explanation for this may relate to the orthogonal nature of the PANAS dimensions, which were devised to be quasi-independent (Watson, 1988). As such, the positive affect subscale does not include many adjectives typically associated with positive mood (e.g., happy, cheerful), but which were perhaps better captured in the other state affect measures included in this trial. We further note that in a recent review we conducted of single session interventions and immunity (Ayling et al., under review), all interventions that showed significant improvements in PANAS subscales were considerably longer ( $\geq 1$  hour) than the intervention examined here (e.g., Kiecolt-Glaser et al., 2008; Kreutz, Bongard, Rohrmann, Hodapp, & Grebe, 2004).

That significant improvements in state affect were observed for the other measures is particularly noteworthy given that participants showed high mean levels of positive affect pre-intervention. This introduced the possibility of a ceiling effect (i.e., limiting the potential for large improvements in state affect). Indeed, it is worth noting that the largest improvements in state affect were observed in participants with higher levels of trait negative affect at baseline. These participants also scored lower on all pre-intervention state affect measures (all  $p$ 's  $<.05$ ). Thus, the observed moderation may simply be due to the fact these individuals had greater scope for their scores to increase following the intervention.

### **Intervention Effects on Non-specific Immunity**

To explore whether the positive mood intervention resulted in any rapid changes to a non-specific aspect of immunity, we measured secretory IgA levels pre- and post-intervention. We observed significant increases in secretory IgA secretion among participants

viewing the positive mood intervention. However, we saw a similar secretory IgA increase in the neutral control group indicating, in this case, that any secretory IgA changes cannot be attributable to a positive mood increase, and is likely best explained as an artefact of the research process common to both groups. Given that PANAS negative affect scores decreased from before to after the intervention in both groups (though this did not reach statistical significance in the control group [ $Z = -1.93, p = .053, r = -.27$ ]) we speculate that the observed increase in secretory IgA levels across groups could reflect some form of relaxation effect. Rest alone has previously been shown to result in increases in secretory IgA levels (Donoyama & Shibasaki, 2010) therefore it may be that just the act of sitting quietly and viewing a 15 minute video was sufficient to result in these changes. Notably, many prior studies reporting an increase in secretory IgA concentration following positive mood induction have adopted cohort or crossover designs without a neutral control group (e.g., Burns, Harbuz, Hucklebridge, & Bunt, 2001; Lefcourt, Davidson-Katz, & Kueneman, 1990). In contrast, studies with control groups similar to ours typically show a similar pattern of findings (e.g., Donoyama, Munakata, & Shibasaki, 2010; Donoyama & Shibasaki, 2010). Collectively, these findings highlight the importance of future studies of secretory IgA and mood including neutral control conditions, to avoid the potentially erroneous attribution of secretory IgA changes to increases in positive mood.

### **Intervention Effects on Vaccine-specific Immunity**

In examining the intervention effects on antibody responses to a quadrivalent influenza vaccination, we observed no statistically significant differences between participants who viewed the positive affect intervention to the neutral control group. This is not surprising given that the trial was not powered for this outcome. Instead our aim was exploratory, in that we sought to determine if there was early evidence of an effect of the intervention on immunity and to explore the feasibility of a subsequent, appropriately

powered trial. In that regard, we observed that for most strains at both 4 and 16 weeks post-vaccination, the point-estimates of effect favoured the positive affect intervention and were small. One interpretation of these findings is that they suggest that the intervention, whilst demonstrating an effect on positive mood, is unlikely to result in an effect on antibody responses of sufficient magnitude to be clinically relevant. But such a conclusion should be interpreted in the context of two further issues. First, it could be argued that any effect in clinical practice could potentially be larger than reported here. Our design included an active neutral control as the comparison arm, rather than usual care (which would have been no stimuli). This may have served to attenuate any observed between group differences on mood and, in turn, on immune outcomes. Second, while we note due caution is warranted in over-extrapolating effect size estimations from pilot trials (Bell & Julius, 2018; Kraemer, Mintz, Noda, Tinklenberg, & Yesavage, 2015; Leon, Davis, & Kraemer, 2011), effect sizes of the magnitude observed here are not necessarily trivial when the intervention is produced at low cost and could be implemented at high volume. Even modest improvements to vaccine-related clinical outcomes could be of substantial benefit to patients and the health service, given the large number of vaccine recipients annually. Indeed, other low-cost high-volume interventions have been widely adopted in clinical practice for the purpose of preventing disease, even where effect sizes are relatively small (e.g., statins for the prevention of major cardiovascular events:  $d = 0.15$ ; Leucht, Helfer, Gartlehner, & Davis, 2015). We propose, therefore, that further research is warranted involving trials in which changes in antibody levels are the primary outcome and the comparator arm is usual care. Only then will we be able to determine definitively if interventions that enhance positive mood are able to enhance vaccine effectiveness and reduce the burden of disease.

In terms of the relationship between positive affect and antibody responses to vaccination more broadly, we found across the entire sample, that higher levels of trait

positive affect were also associated with significantly larger antibody responses to the influenza vaccination (at least for the B strains of the vaccine). This replicates prior findings demonstrating associations between trait, or long-term, positive affect and antibody responses to vaccination (Ayling et al., 2018; Marsland et al., 2006). Mechanisms underpinning this association continue to be elucidated and likely include both direct effects of the central nervous system on immune function (Marques-Deak et al., 2005; Pressman & Cohen, 2005) and indirect pathways. Whether these pathways can be leveraged to improve vaccine-related outcomes in older adults remains a further avenue for researchers to explore.

### **Feasibility of a Larger Trial**

This study supports the feasibility of conducting a future larger trial of the intervention, potentially including an examination of effects on non-surrogate clinical endpoints. The vast majority of participants receiving their allocated intervention as intended, and high retention rates were observed across all follow-ups. While the uptake rate (9.4%) in this trial was consistent with our prior studies in this area (Ayling et al., 2018) that have also relied on a low-intensity recruitment approach (i.e., single invitation letter), there remains the potential to improve these uptake rates with a more intensive approach. Evidence based strategies for boosting recruitment rates which could be considered include telephone reminders to non-responders and in-person patient screening and recruitment approaches (Ngune, Moyez, Dadich, Lotriet, & Sriram, 2012; Treweek et al., 2018). Implementing such strategies would likely be beneficial in increasing uptake rates in a future larger trial.

### **Limitations and Strengths**

It is important to acknowledge that our results here relate to a quadrivalent egg-derived influenza vaccine, and we do not know whether these findings will generalise to future vaccines in an evolving landscape of approved influenza vaccines for use in older adults. For example, the 2018/19 influenza vaccination season saw the introduction of an

adjuvanted trivalent vaccine in the NHS (Wise, 2018), and the European Medicine Agency (2018) recently approved cell-based quadrivalent and high-dose egg-derived trivalent vaccines for use in 2019/20. Further research is needed to establish whether any benefits of a brief positive mood intervention prior to vaccination are evident with different vaccine formulations.

We also note that our sample were self-selecting, and therefore may not be entirely representative of the older adult population. For the majority of demographic variables assessed, we were successful in recruiting across a broad spectrum of older adults. However, one concern is the lack of ethnic diversity of the recruited sample, which was almost exclusively made up of those who identified as White-British. Moving towards a larger trial, the adoption of recommendations for increasing ethnic minority adults into clinical research (e.g., Areán, Alvidrez, Nery, Estes, & Linkins, 2003; Areán & Gallagher-Thompson, 1996) will need to be considered.

This is the first trial to examine the effects of a brief positive mood intervention administered prior to a quadrivalent influenza vaccine in primary care. We adopted a controlled, single-blinded study design and made attempts to minimise the effects of demand characteristics, by concealing aspects of the research from participants, namely by not highlighting there were two groups, one of which was a neutral control. However, participants were aware the study was examining the relationship between mood and influenza vaccination responses. As such, we cannot rule out the possibility that the observed effects on self-reported mood outcomes are influenced by this knowledge. Relatedly, while steps were taken to keep researchers blind to participant allocation, due to common reactions of participants to the positive mood intervention (e.g., laughter) it is possible that researchers could have inferred group assignment for some participants. As the main outcome measures were either participant reported immediately post-intervention (state affect), or machine-read

optical absorbance values (immune), any lack of researcher blinding is unlikely to have influenced the study findings.

### **Concluding Remarks**

A brief 15-minute intervention resulted in significant improvements in positive mood in older adults prior to receiving their annual influenza vaccination. Point-estimates of effects on antibody responses indicated that effects on antibody were in the predicted direction. Further research is now needed to establish whether a brief intervention could act as a low-cost, high volume method for enhancing the effectiveness of influenza vaccination in older people.

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Figure 1: Flow Diagram of Participants

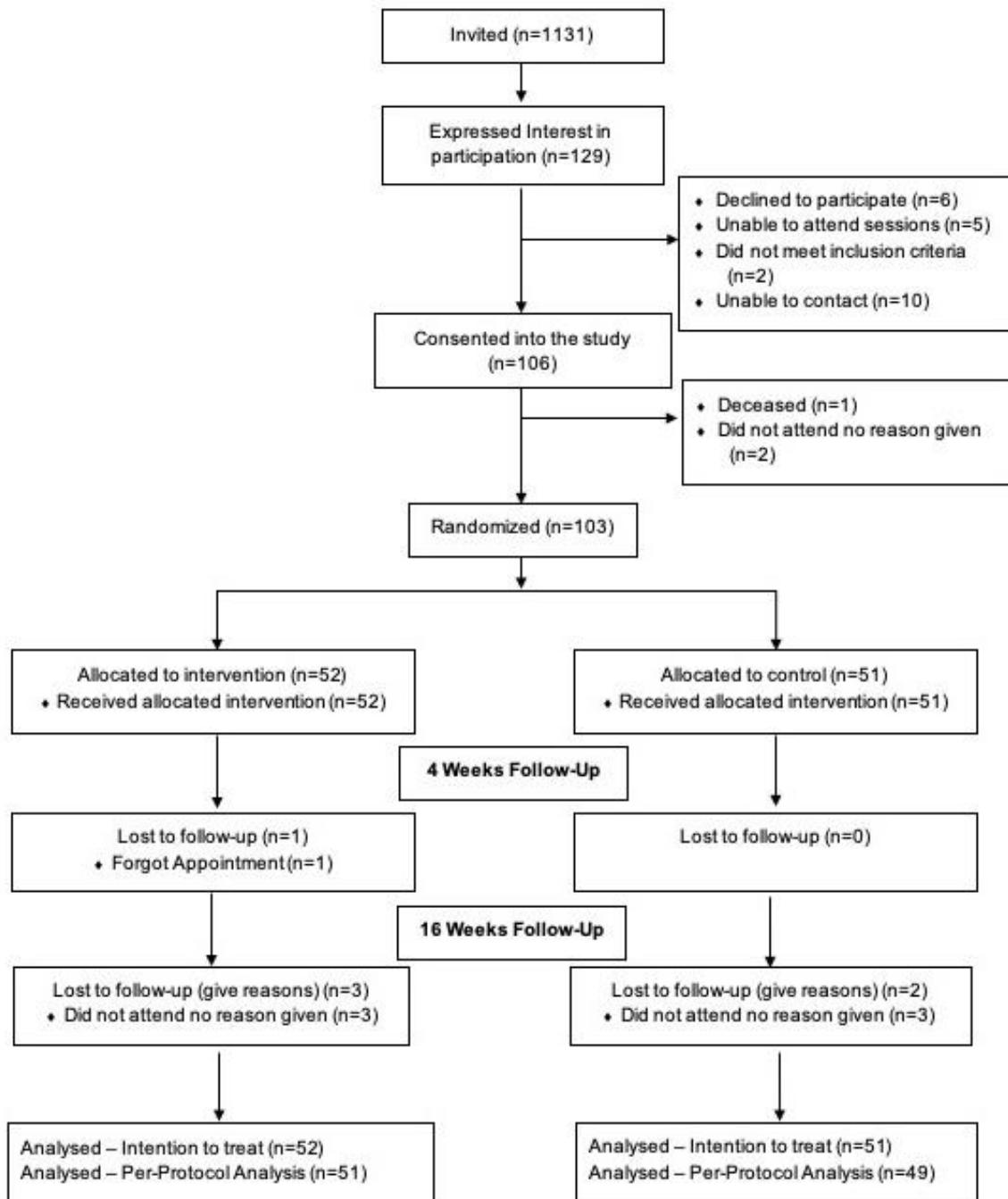


Figure 2: Antigen specific IgG responses to vaccination by group. Bars are antilog transformed pre-vaccination IgG adjusted-means. Error bars are 95% confidence intervals.

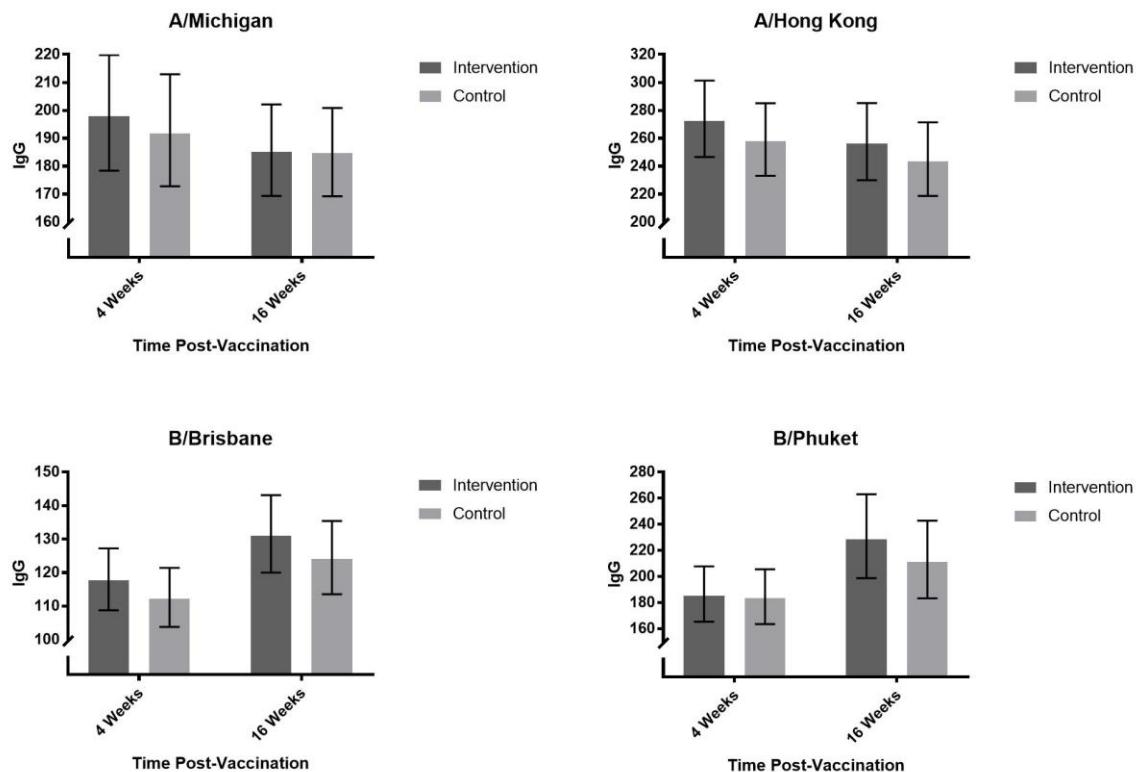


Table 1: Participant demographics by study arm<sup>+</sup>

Variable	Positive Mood Intervention	Neutral Control	Test for Between-Condition Differences
Age - yr	72.6 (4.6)	73.3 (5.1)	$t(100) = 0.71, p = .480$
Female sex – n (%)	22 (42.3)	30 (58.8)	$\chi^2(1) = 3.19, p = .074$
White Ethnicity – n (%)	51 (98.1)	48 (94.1)	$\chi^2(2) = 1.05, p = .591$
Marital Status – n (%)			$\chi^2(4) = 6.57, p = .161$
Married	34 (65.4)	29 (56.9)	
Single, never married	0 (0)	3 (5.9)	
Separated/divorced	9 (17.3)	4 (7.8)	
Widowed	4 (7.7)	8 (15.7)	
Cohabiting	3 (5.8)	3 (5.9)	
Did not respond	2 (3.8)	4 (7.8)	
Highest Level of Education – n (%)			$\chi^2(3) = 3.08, p = .380$
School	37 (71.2)	40 (78.4)	
Undergraduate	2 (3.8)	0 (0)	
Postgraduate	0 (0)	1 (2.0)	
Other	8 (15.4)	8 (15.7)	
Did not respond	5 (9.6)	2 (3.9)	
Living Independently – n (%)	47 (90.4)	49 (98.0)	$\chi^2(1) = 2.67, p = .102$
Highest Ever Total Household Income – n (%)			$\chi^2(4) = 1.80, p = .773$
≤ £14,999	17 (32.7)	16 (31.4)	
£15,000–£24,999	16 (30.8)	17 (33.3)	
£25,000–£34,999	8 (15.4)	4 (7.8)	
£35,000–£49,000	7 (13.5)	5 (0.8)	
£50,000–£74,999	1 (1.9)	2 (3.9)	
£75,000–£99,000	0 (0)	0 (0)	
≥ £100,000	0 (0)	0 (0)	
Did Not Respond	3 (5.8)	7 (13.7)	
Current Smoker – n (%)	4 (7.7)	4 (7.8)	$\chi^2(1) = 0.003, p = .954$
Total Health Status	555.7 (175.1) <sup>1</sup>	555.1 (169.9)	$t(95) = -0.02, p = .988$
Trait Positive Affect (PANAS)	33.5 (8.6)	33.8 (8.1)	$U = 1104.5, Z = -.15, p = .878$
Trait Negative Affect (PANAS)	15.2 (6.7)	15.5 (6.4)	$U = 1013.5, Z = -.32, p = .753$
Trait Optimism	16.0 (4.8)	15.3 (4.5)	$t(94) = -.80, p = .424$
Trait Emotional Reactivity	39.9 (17.2)	41.3 (16.0)	$t(93) = .71, p = .481$
Pre-Vaccination IgG			
A/Michigan	187.2 (112.4)	156.6 (103.1)	$t(101) = -1.84, p = .069^{\#}$
A/Hong Kong	238.0 (119.9)	241.0 (158.1)	$t(101) = -0.46, p = .646^{\#}$
B/Brisbane	107.0 (54.1)	118.9 (78.7)	$t(92.32) = 0.19, p = .850^{\#}$
B/Phuket	144.4 (85.1)	183.2 (146.2)	$t(101) = 1.01, p = .313^{\#}$

<sup>+</sup>Mean (standard deviation) unless otherwise specified<sup>#</sup>Based on log-transformed values

Table 2: Pre- and Post-Intervention Momentary Affect and S-IgA scores by group [Mean (SD)]

<b>Variable</b>	<b>Positive Mood Condition(n=52)</b>		<b>Neutral Control Condition (n=51)</b>	
	Pre-Intervention	Post-Intervention	Pre- Intervention	Post- Intervention
Slider-valence	79.5 (19.2)	87.4 (15.7)	80.5 (18.1)	79.8 (20.8)
Slider-arousal	79.9 (19.0)	83.7 (16.8)	80.9 (20.1)	79.8 (20.3)
Pictorial	3.7 (1.5)	4.7 (1.1)	4.0 (1.5)	4.0 (1.5)
Positive Affect (PANAS)	34.3 (7.3)	35.0 (8.4)	34.6 (7.2)	35.0 (7.6)
Negative Affect (PANAS)	12.3 (4.0)	11.1 (3.0)	12.3 (4.0)	11.4 (2.3)
Salivary IgA ( $\mu\text{g}/\text{min}$ )	81.0 (76.4)	116.7 (107.5)	94.1 (94.2)	144.7 (142.5)

<sup>+</sup>Based on positive mood condition: n=46; neutral control condition: pre-intervention n=42, post intervention n=41