**Salivary functional antibody secretion is reduced in older adults: a potential mechanism of increased susceptibility to bacterial infection in the elderly**

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**Running title:** Functional antibodies and age

**Abstract**

**Background:** Bacterial infections in the elderly are common and associated with high morbidity and mortality, with pneumonia the second commonest cause of death. Reductions in antibodies against specific bacterial antigens in saliva and serum could contribute to infection risk in older adults, although they have yet to be examined in relation to age.

**Method:** IgG, IgA and IgMantibody levels in paired saliva and serum samples were measured against 12 pneumococcal , 4 meningococcal and haemophilus polysaccharide antigens and diphtheria and tetanus toxoids in healthy younger (n = 28, 21–34 years) and older (n = 44, 60–80 years) adults.

**Results:** Older adults had lower antibody concentrations in saliva than young adults, with the most striking differences observed for salivary antibody secretion rates. In serum, older adults registered lower concentrations for only a minority of antibodies. Young adults who had previously received a polysaccharide pneumococcal vaccination (PPV23) had higher levels of anti-pneumococcal antibodies in serum and in saliva. Only minor differences were observed in antibody levels between older adults who had/had not received PPV23, and there was no evidence of memory in saliva.

**Conclusions:** Age differences were much greater in salivary antibodies than in serum; older adults had reduced salivary secretion rates of antibodies across bacterial antigens. This decline in local immunity may contribute to increased infection risk in the elderly. The poor memory from pneumococcal vaccination in serum and saliva suggests that PPV23 may be ineffective in older adults for both systemic and local protection.

**Introduction**

Age-related immune decline and dysregulation, immunosenescence, is associated with increased susceptibility to bacterial and viral infections ([1](#_ENREF_1)). Infectious diseases in the elderly are more common and severe than in younger adults ([2](#_ENREF_2)), with pneumonia being the second commonest cause of death in over 75s and community acquired pneumonia (CAP) accounting for a fifth of all deaths ([3](#_ENREF_3), [4](#_ENREF_4)). *Streptococcus pneumoniae* (*S pneumoniae*) causes up to half of CAP and is a commensal bacteria of the upper respiratory tract (URT) but can spread to other areas and result in invasive disease ([5](#_ENREF_5)). Most cases of bacterial meningitis can be attributed to *S pneumoniae*, which is associated with earlier and higher mortality rates in older adults ([6](#_ENREF_6), [7](#_ENREF_7)). Elderly individuals are also at risk of bacterial infection due to *neisseria meningitides*; generally manifesting as pneumonia rather than meningitis ([8](#_ENREF_8)). Further, adults aged over 65 years who contract a disease caused by *haemphilus influenzae* bacteria are at higher risk of mortality than young adults ([9](#_ENREF_9)). Therefore, vulnerability to, and severity of, bacterial infections in older adults is evident across a range of bacteria.

Immunosenescence in the URT has been suggested to underlie the increased susceptibility of older adults to pneumococcal disease ([5](#_ENREF_5)). Salivary antibodies play an important role as the first line of defence against pathogens and assist in controlling carriage of bacteria, such as *S pneumoniae* and *haemphilus influenzae*, and consequently respiratory and invasive disease ([10](#_ENREF_10)). Accordingly, changes in salivary antibodies with ageing may contribute to mucosal immunosenescence and infection risk in older adults.

Immunoglobulin A (IgA), in its secretory form, is the main class of antibodies in saliva and IgA concentration and secretion rate has been used as a marker of mucosal immunity and to assess risk of URT infection ([11](#_ENREF_11)). Salivary IgA secretion rates have been shown to be significantly lower in elderly individuals and decrease with increasing age ([12](#_ENREF_12), [13](#_ENREF_13)). Conversely, in parotid saliva, IgA, IgG and IgM concentrations were reported to be significantly higher in the elderly ([14](#_ENREF_14)). However, this study did not consider flow or secretion rates, which influence concentrations of saliva components, including antimicrobial proteins ([15](#_ENREF_15)). Further, antibodies in whole saliva may differ from the specific glands, especially for IgA as the density of IgA positive plasma cells can vary between each salivary gland ([16](#_ENREF_16)). Whole saliva can provide an overview of production from the various glands in the mouth.

Few have investigated functional antibodies in saliva to provide an insight into protection against specific bacteria in relation to ageing. One exception demonstrated age-associated reductions in IgG and IgM saliva secretion rates specific to *streptococcus mutans*, *escherichia coli* and *actinomyces viscous* ([17](#_ENREF_17)). Age-related changes to these commensals may differ to pathogenic bacteria, however, the effect of age on salivary antibodies specific to bacteria that pose a threat of infection, including *S pneumoniae*, *neisseria meningitides* and *haemphilus influenza* has yet to be explored.

Evidence suggests that systemic functional antibodies are reduced in the elderly. Concentrations of naturally acquired IgG antibodies in serum against pneumococcal serotypes (Pn) 3 and 6B and IgM antibodies against Pn 3, 4, 6B, 9V and 23F have been shown to be reduced in those aged 65+ years ([18](#_ENREF_18)). However, serum has not been measured in parallel with saliva across a range of bacteria.

To determine whether salivary antibodies against specific bacterial antigens are reduced in older adults, and potentially a mechanism contributing to compromised immunity and infection risk, the present study examined functional antibodies against 19 different antigens in healthy younger and older adults. Paired saliva and serum samples were analysed to compare local and systemic protection.

**Methods**

*Participants*

Participants were 44 community dwelling older adults (21 females) aged 60–80 years (mean = 66.8, SD = 4.8) and 28 (13 females) postgraduate students aged 21–34 years old (mean = 22.8, SD = 2.5). Seven (17%) of older participants reported suffering from a chronic illness: 4 participants had hypertension (one of which also reported asthma), 1 had COPD, 1 had benign prostatic hypertrophy and 1 suffered from trigeminal neuralgia. Among older adults, 30% reported taking medication: anti-hypertensives, non-corticosteroid inhalers, statins, and gastrointestinal medications. Younger adults had no chronic illness/medication usage. No participants were suffering from any acute illness 2 weeks prior to, or during the study. Key exclusion criteria included gum disease, or any teeth/oral problems.

*Serum and saliva collection*

Participants were asked to refrain from exercise and alcohol 24 h prior, and food and caffeine 12 h prior to arriving at the laboratory between 7–9am. Their vaccination history was recorded. A 6ml venous blood sample and an unstimulated whole-saliva sample were collected (further details are described in Supplementary Methods).

*Functional antibody measurements*

Saliva and serum were analysed using a multi-plexed bead assay (Luminex-200, Bio-plex systems, BioRad Laboratories, California, USA) that simultaneously measured antibody titres against: 12 pneumococcal (Pn) (serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F), 4 meningococcal (MEN) (serotypes A, C, W-135 and Y), tetanus (TET), diphtheria (DIP) and haemophilus (HIB) antigens. Assay reproducibility, linearity and specificity are described in Supplementary Methods. Serum and saliva samples were assayed x 3 to measure antibodies specific to IgG, IgM and IgA. Concentrations for IgM and IgA specific antibodies for meningococcal serotypes, TET, DIP and HIB were not obtained, as values for these antigens for the reference sera (89SF) used in the 19-plex assay are not currently available ([19](#_ENREF_19)).

*Data Analysis*

For saliva, age differences in antibody concentrations, salivary flow rate and antibody secretion rate were analysed. Salivary flow rate (mL/min) was calculated as volume of saliva/collection time. Saliva secretion rates are reported to reflect the total availability of immunoglobulin at the oral surface and control for hydration status ([20](#_ENREF_20)). Secretion rates (ng/min) were calculated as saliva flow rate x antibody concentration. For serum, age differences in functional antibody concentrations were examined. Antibody parameters were explored for each functional antibody for IgG, IgA and IgM, respectively. Mann Whitney U-tests were used to compare antibody parameters between: age groups, pneumococcal vaccination status within age groups, and older adults who were/were not taking on-going medication. Where significant differences were observed, *p* values were re-assessed using the Holm-Bonferroni method to control for the multiple comparisons being made for the number of dependent variables generated from using a multiplex assay for each immunoglobulin class. For IgG protective titres were: 0.35 μg/mL for Pn antibody serotypes,([21](#_ENREF_21)) 2 μg/mL for Men antibody serotypes ([22](#_ENREF_22)), 1.0 μg/mL (long-term) and 0.15μg/mL (short-term) for HIB antibody ([23](#_ENREF_23)), and 0.1 IU/mL (long-term) and 0.01 IU/ml (short-term) for TET and DIP antibody ([24](#_ENREF_24)).

**Results**

*Age and functional antibodies in saliva*

Compared with younger adults, older participants registered significantly lower concentrations of functional antibodies in saliva for several antibodies: 3/12 for IgA; 4/12 for IgM; 4/12 for IgG Pn antibodies and also for IgG, 4/4 Men antibodies, DIP and HIB (Table 1). No difference was seen between age groups for TET. Following Holm-Bonferroni correction, differences that were initially indicated significant at the 0.05 level failed to remain significance. Although not statistically significant, older adults had a lower salivary flow rate than younger adults, with median rates of 0.31 mL/min (range, 0.10–0.97 mL/min) and 0.38 mL/min (range, 0.11–0.89 mL/min), respectively. Older adults had lower functional antibody saliva secretion rates than younger adults for the 25/36 comparisons of 12 Pn serotypes across IgG, IgM and IgA (Figure 1). Figure 2 shows this age difference in saliva secretion rates was also evident for IgG MEN serotypes, TET, DIP and HIB. Following Holm-Bonferroni correction, differences in secretion at the 0.05 level were no longer significant across all immunoglobulin subclasses, in addition to Pn9V for IgM.

*Age and functional antibodies in serum*

As presented in Table 2, older adults demonstrated lower concentrations for fewer antibodies than observed in saliva; 1/12 for IgA; 3/12 for IgM; 2/12 for IgG Pn antibodies, and also 3/4 MEN, HIB and DIP for IgG (not TET). Only findings with *p* values indicated as ≤ 0.01 withstood adjustment.

*Antibody levels in relation to vaccination and protective titres*

Ten participants (36%) in the younger adult group had received PPV23 within the past 4 years through research participation, and a minority had received other vaccinations relevant to this investigation: meningitis C (n = 5), tetanus (n = 3). Fifteen older adults (34%) had received PPV23 within the past 6 years.

In saliva, younger adults who had received PPV23 had higher concentrations of 5 IgG and 2 IgA specific Pn serotypes compared to those who had not been vaccinated (Supplementary Table 1, S1). The same significant findings emerged for salivary secretion rates; with the addition of Pn5, 7, 19A and 23F for IgG and Pn3 for IgA, all *p* < .05. However, these vaccination differences in saliva did with not withstand Holm-Bonferroni adjustment. In older adults, no significant differences were observed in relation to vaccination for salivary antibody concentrations (Table S1) or secretion rates.

In serum, younger adults who had received PPV23 demonstrated higher antibody concentrations for IgG (8/12), IgA (12/12) and IgM (5/12) serotypes (Supplementary Table 2). The serum levels of anti-Pn Ig of vaccinated and non-vaccinated older adults were observed for fewer serotypes: IgG (4/12), IgA (5/12) and IgM (1/12). Findings indicated as significant at the 0.05 level, for both younger and older vaccination groups, were not upheld following correction for multiple comparisons. In general, vaccinated younger adults had higher antibody concentrations compared with vaccinated older adults.

In younger adults, 68% demonstrated protective levels on ≥ 8/12 Pn serotypes, and 66% of older adults displayed protective levels on ≥ 8/12 serotypes. For younger adults, protective titres were observed in 54%, 26%, 7% and 32% of individuals for Men A, C, W and Y, respectively. A similar proportion of older adults were protected against Men A (52%), although no older participants were protected against Men C or W and only 9% against Men Y. All, or almost all, younger adults were protected against Tet (100%), DIP (96%) and HIB (82%), with the majority of individuals reaching levels for long-term protection. In older adults, 91% were protected against TET, 57% against DIP and 59% against HIB.

*Chronic medication*

In older adults, those taking chronic medication had significantly lower salivary IgM antibodies for Pn1, 4, 5, 9, 18C, 19A, 19F and 23F compared with individuals who were not, *p* < 0.05 for all comparisons. A similar pattern emerged for saliva secretion rates, where Pn1, 5, 9, 18C, 19A, 19F and 23F secretion was significantly (*p* < 0.05) reduced for IgM. There were no significant differences for IgG or IgA, secretion rates, or flow rate. In serum, older adults taking chronic medication had significantly lower levels of IgM against Pn 19F and 23F. Findings in relation to chronic medication did not reach *p* < 0.01, as such they do not remain significant when applying Holm-Bonferroni adjustment to observed *p* values. There was no significant difference in age in older adults between those who were and were not taking medication.

**Discussion**

This is the first study that has carried out bacterial functional antibody analyses of this depth in relation to age. Secretion of salivary antibodies against bacterial antigens was significantly reduced in older adults. It has previously been shown that IgG and IgM salivary antibody secretion rates for commensal bacteria are reduced with age ([17](#_ENREF_17)); the present study extends these findings to bacteria that cause infectious disease. Antibody saliva concentrations were also significantly lower in older adults, although findings were less consistent compared with secretion. Secretion may be a more relevant as it takes into account changes in saliva volume and, for IgA, it has been proposed the combination of concentration and flow reflects the total amount of immunoglobulin covering the mucosal surface, thus providing a better estimate of protection ([11](#_ENREF_11)). Indeed, secretion rate, rather than concentration of salivary IgA has been found to be the best predictor of URT infection ([15](#_ENREF_15)). The reduced antibody secretion rate observed in saliva of older adults reveals another aspect of immunosenescence that may contribute to infection risk in the elderly.

Although older adults had lower flow rates than their younger counterparts, the difference was not significant; this is consistent with some previous findings others ([25](#_ENREF_25)) but not others ([13](#_ENREF_13), [26](#_ENREF_26)). Dry mouth, xerostomia, is common in older adults and is associated with decreased saliva flow ([25](#_ENREF_25), [26](#_ENREF_26)) and an increased incidence of infections ([27](#_ENREF_27)). Reduced saliva flow rates in the elderly have been proposed to be driven by medication use ([26](#_ENREF_26)). We found that older adults taking chronic medication had lower concentrations and secretions of salivary IgM, but not IgA or IgG antibodies. These findings should be interpreted cautiously, as they did not withstand statistical adjustment. Due to the small sample size, in addition to the low proportion of older adults taking medication (perhaps not representative of the wider elderly population), the effects of medication on IgM specific antibodies should be considered preliminary findings and the effects of medication, and also chronic disease/types of disease, on salivary anti-bacterial antibodies requires further investigation. Interestingly, some of the medications reported included dry mouth as a potential side effect, yet no difference in saliva flow rates were observed between those who were and were not taking medication. Studies have shown that flow rate is not affected by age in healthy individuals who are not taking medication ([25](#_ENREF_25)). However, in the present study, older adults who were not taking medication also had lower secretion rates than younger adults. This suggests that the reduction in secretion rate may be due to ageing *per se*, and not simply reflecting reduced flow rate due to medication.

Results in serum did not mirror those of saliva, with lower concentrations in older adults only being observed for a few antibodies. Previous findings have illustrated a reduction in naturally acquired IgG and IgM in older adults against certain pneumococcal serotypes ([18](#_ENREF_18)). Interestingly, the majority from both age groups demonstrated protective levels on ≥ 8 of the 12 Pn serotypes, yet ≤ 35% had been vaccinated. Implying that in healthy individuals, high proportions achieve protective levels through natural exposure. However, protection against bacteria may depend on antibody function and not just antibody availability: deficits in opsonophagocytic activity and avidity may contribute to risk of pneumococcal infection in the elderly ([5](#_ENREF_5)).

Younger adults who had received PPV23 had higher serum antibody concentrations across immunoglobulins and serotypes, whereas minimal differences were observed between vaccinated and unvaccinated older adults. Younger adults who had been vaccinated had higher antibody concentrations than vaccinated older adults, suggesting an age-related deterioration in the primary response and/or memory. Although this may support impaired vaccination efficacy of PPV23 with ageing highlighted previously ([28](#_ENREF_28)), this cannot be determined in this cross-sectional study. The PPV23 programme in the UK for those aged > 65 shows little impact on incidence rates of invasive pneumococcal disease (IPD) ([29](#_ENREF_29)). The seven-valent pneumococcal conjugate vaccine (PCV7) was integrated into UK routine childhood immunisation programme in 2006 and replaced by a thirteen-valent equivalent (PCV13) in 2010. This resulted in both direct and indirect population protection via herd immunity and reduced bacterial carriage, evidenced by large reductions in IPD from vaccine specific serotypes ([30](#_ENREF_30)). A recent trial evaluating the efficacy of PCV13 in adults aged ≥ 65 (CAPiTA) has shown significant reductions in CAP and IPD ([31](#_ENREF_31)). Although PCV13 appears to be more effective in older adults than PPV23, it is also much more expensive.

In younger adults who had received a pneumococcal vaccine, in addition to higher IgG, increased antibodies for IgM and IgA were also observed. In a prior study involving healthy individuals aged 22–55 years vaccinated with PPV23, pneumococcal polysaccharide specific IgM and IgA was shown to remain higher than pre-vaccination levels one year later ([32](#_ENREF_32)). In the present study, younger adults who received a vaccination had done so within two years, which may account for elevated IgM and IgA levels.

In saliva, younger adults who had received PPV23 showed preliminary evidence of higher IgG and IgA concentrations and secretion rates than those not vaccinated. In contrast, no differences were observed in relation to saliva and vaccination for older adults, suggesting PPV23 is ineffective in producing local memory in this age group. In children, significant increases in salivary antibodies have only been shown following a booster vaccination ([33](#_ENREF_33)). It may be that a threshold concentration needs to be reached in serum for protection to be reflected at a local level, or booster vaccinations are required in order to elicit a mucosal response; however, re-immunisation with pneumococcal polysaccharide vaccines can result in immune hyporesponsiveness ([34](#_ENREF_34)). Alternatively, any salivary response may have diminished over time. An increase in salivary antibodies has been observed in adults seven days following a pneumococcal vaccine, but maintenance over time was not explored ([35](#_ENREF_35)). The present findings suggest that PPV23 cannot confer memory in saliva of older adults, although further studies specifically looking at vaccination are required.

The proportions of participants with protective levels for MEN serotypes, TET, DIP and HIB were similar to those observed previously in healthy adults ([19](#_ENREF_19)), although reference ranges for age have not been established. Reference ranges are not available for saliva, although this study has demonstrated that is it possible to detect these 19 bacterial antigen specific antibodies in saliva.

In conclusion, older adults have reduced salivary secretion rates of IgA, IgM and IgG antibodies against a range of antigens. Therefore, the reduction in adaptive immunity observed with ageing appears to extend to secretory oral immunity. This decline in local protection may contribute to increased susceptibility to infection in the elderly. A relatively small sample size is a limitation of this investigation and findings should be replicated in larger studies. The small sample size was a likely contributor in findings to remain significant at the 0.05 level following adjustment, although all findings at 0.01 and 0.001 were still supported following correction for multiple comparisons. To understand the practical implications of age-related changes in oral functional antibodies, future studies should explore the relationship between saliva parameters, carriage of bacteria and infection incidence. Given that the transmission of pneumococci may occur via saliva and saliva swabs have been proposed as a method to improve detection of pneumococcal colonisation ([5](#_ENREF_5), [36](#_ENREF_36)), saliva may become of increasing importance in research relating to infectious disease in the elderly.

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**Disclosures**

The authors report no conflict of interests

**Ethics**

All participants gave written informed consent prior to the study, which had the appropriate Ethics Committee approval.

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**Table 1.** IgG, IgM and IgA functional antibody concentrations for young and older adults in saliva. Data presented is median (5–95 percentile) in ng/ml. Significant differences between young and old are indicated by \*\*\* *p* < .001, \*\* *p* < .01, \* *p* < .05

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| --- | --- | --- | --- |
| **Saliva** | **IgG** | **IgM** | **IgA** |
|  | **Young** | **Old** | **Young** | **Old** | **Young** | **Old** |
| Pn1 | 1.10(0.23–7.47) | 1.03(0.13–8.59) | 3.24(0.79–17.27) | 2.44(0.09–7.98) | 3.75(0.99–27.51) | 5.50(0.86–37.32) |
| Pn3 | 0.38\*\*\*(0.83–3.78) | 0.2(0.06–0.69) | 0.47(0.08–10.40) | 0.39(0.03–2.93) | 58.67\*\*(9.83–230.23) | 27.83(5.89–142.37) |
| Pn4 | 0.35\*(0.10–3.13) | 0.24(0.04–1.52) | 1.71\*(0.41–10.15) | 1.03(0.05–5.70) | 15.48(2.92–174.48) | 10.5(2.36–35.13) |
| Pn5 | 1.30(0.26–53.42) | 1.10(0.14–34.29) | 5.27(1.62–57.61) | 3.99(0.21–15.63) | 3.24(0.58–20.55) | 2.75(0.76–9.22) |
| Pn6B | 0.59(0.08–7.24) | 0.56(0.10–13.19) | 10.46(2.19–59.68) | 9.72(0.43–32.36) | 13.03\*(1.37–63.12) | 7.7(1.32–70.66) |
| Pn7F | 2.63(0.38–15.16) | 1.61(0.21–17.06) | 2.21(0.76–14.61) | 1.83(0.15–6.44) | 7.56(1.90–30.68) | 5.44(1.00–28.92) |
| Pn9V | 0.96(0.13–9.30) | 0.54(0.08–3.64) | 3.37(1.22–23.65) | 3.27(0.21–11.12) | 16.57\*(2.34–106.53) | 6.66(0.92–42.55) |
| Pn14 | 2.94\*\*(0.17–83.04) | 0.74(0.13–14.24) | 2.77(0.20–52.15) | 0.76(0.20–39.34) | 31.71(5.72–290.31) | 22.52(3.73–230.78) |
| Pn18C | 0.43(0.03–33.42) | 0.54(0.09–9.09) | 1.47(0.30–9.78) | 1.05(0.05–11.49) | 7.73(0.71–42.08) | 4.45(0.69–32.86) |
| Pn19A | 7.07(1.78–27.67) | 4.92(1.00–38.94) | 4.26(1.26–25.98) | 3.2(0.31–12.90) | 23.9(12.07–282.96) | 20.16(5.74–70.79) |
| Pn19F | 7.10\*\*\*(2.31–53.65) | 3.53(0.64–20.55) | 23.67\*\*\*(4.78–103.02) | 10.4(0.48–41.30) | 32.07(14.49–88.79) | 27.76(6.39–84.37) |
| Pn23F | 0.49(0.05–44.45) | 0.51(0.04–3.45) | 7.18\*(2.10–52.58) | 5.58(0.38–17.01) | 10.76\*(1.16–64.72) | 5.18(1.22–29.57) |
| MenA | 2.48\*\*(0.45–19.11) | 1.27(0.26–6.92) |  |  |  |  |
| Men C | 0.38\*\*\*(0.05–12.20) | 0.07(0.05–0.44) |  |  |  |  |
| Men W-135 | 0.41\*\*\*(0.12–7.79) | 0.23(0.07–0.60) |  |  |  |  |
| MenY  | 1.63\*\*\*(0.23–13.83) | 0.6(0.14–1.39) |  |  |  |  |
| Tet | 0.64(0.07–111.26) | 0.3(0.03–28.37) |  |  |  |  |
| Dip | 0.46\*\*\*(0.03–25.81) | 0.06(0.01–0.92) |  |  |  |  |
| Hib  | 0.56\*(0.26–9.12) | 0.4(0.21–8.11) |  |  |  |  |

**Table 2.** IgG, IgM and IgA functional antibody concentrations for young and older adults in serum. Data presented is median (5–95 percentile) in ng/ml. Significant differences between young and old are indicated by \*\*\* *p* < .001, \*\* *p* < .01, \* *p* < .05

|  |  |  |  |
| --- | --- | --- | --- |
| **Serum** | **IgG** | **IgM** | **IgA** |
|  | **Young** | **Old** | **Young** | **Old** | **Young** | **Old** |
| Pn1 | 299.34(51.59–12953.36) | 304.38(44.16–6412.99) | 281.23(103.03–3415.12) | 312.33(90.01–1013.82) | 103.59(9.45–1237.62) | 190.42(27.29–639.19) |
| Pn3 | 582.37\*(19.21–12941.55) | 152.27(32.11–11110.47) | 632.70(68.13–19003.92) | 389.69(55.32–6890.76) | 845.25\*(82.87–9692.91) | 467.82(127.38–3803.65) |
| Pn4 | 195.98(48.42–3755.09) | 277.41(25.45–4746.92) | 337.86(99.91–3561.74) | 333.74(70.31–3026.05) | 138(18.66–3052.98) | 151.64(12.68–4338.53) |
| Pn5 | 951.68(159.19–12014.62) | 1114.98(241.43–23670.73) | 1514.88(386.87–5208.61) | 1102.92(263.26–2496.30) | 80.41(10.16–958.48) | 78.65(15.86–871.71) |
| Pn6B | 427.51(22.76–44249.30) | 359.68(56.06–7109.07) | 1064.67(147.36–7960.65) | 1142.21(171.25–4984.58) | 77.54(12.73–956.44) | 142.74(11.28–781.02) |
| Pn7F | 1977.86(245.10–13805.05) | 1419.26(166.84–17948.93) | 771.69(186.16–3851.59) | 481.87(133.51–1594.05) | 123.80(21.49–4730.28) | 146.86(17.83–2774.45) |
| Pn9V | 853.12(58.82–15344.72) | 733.19(80.95–7960.02) | 948.64(295.08–3827.59) | 894.71(144.18–2478.06) | 176.84(15.20–2590.03) | 116.74(21.61–1660.22) |
| Pn14 | 1068.38(31.39–25671.59) | 1112.29(102.98–16161.97) | 490.06\*(86.09–4013.94) | 230.83(36.80–3278.58) | 112.92(22.71–3742.41) | 140.20(40.44–2340.90) |
| Pn18C | 755.57(97.04–13749.16) | 1907.03(37.95–13928.89) | 367.58(95.95–2818.18) | 374.23(69.83–2125.18) | 75.71(11.79–791.46) | 68.38(14.57–431.15) |
| Pn19A | 2253.72(551.33–13255.62) | 1622.64(420.69–16304.13) | 890.01(337.35–6160.14) | 944.32(218.02–3224.72) | 446.13(189.63–3014.70) | 382.79(155.38–8635.27) |
| Pn19F | 2363.60\*(595.28–25555.95) | 1303.83(247.98–8035.79) | 2724.66(669.59–13556.70) | 2461.9(619.23–9363.67) | 402.05\*(100.87–2165.90) | 208.73(85.47–617.80) |
| Pn23F | 412.85(27.48–8927.10) | 549.87(56.30–10487.94) | 931.82(274.73–6977.14) | 913.75(183.05–3617.96) | 78.39(19.50–1125.66) | 78.15(15.37–460.81) |
| MenA | 2380.68(356.64–90154.76) | 2260.4(402.00–16423.32) |  |  |  |  |
| Men C | 395.56\*\*(5.73–9457.28) | 16.75(4.54–479.56) |  |  |  |  |
| Men W-135 | 234.88\*\*(41.71–16646.29) | 99.07(22.90–268.29) |  |  |  |  |
| MenY  | 814.21\*\*(117.85–23934.41) | 354.03(95.19–4942.57) |  |  |  |  |
| Tet | 2004.43(272.67–19458.13) | 1561.42(6.05–17722.37) |  |  |  |  |
| Dip | 158.33\*(13.41–14067.30) | 17.19(1.11–135.57) |  |  |  |  |
| Hib  | 569.91\*(111.64–3718.87) | 171.46(41.89–8759.32) |  |  |  |  |

**Figure legends**

**Figure 1.** Specific IgA (A) IgM (B) and IgG (C) antibody saliva secretion rates for 12 pneumococcal (Pn) antigens in younger and older adults. Boxes represent the 25–75th percentile, with the line indicating the median, and whiskers show the 5–95 percentile. For the majority of Pn serotypes, younger adults had higher antibody secretion rates. Significance is indicated by \**p* < 0.05; \*\**p* < 0.01, \*\*\**p* < 0.001

**Figure 2.** Specific IgG antibody saliva secretion rates for meningococcal (A), haemophilus (B) tetanus and diphtheria (C) antigens in younger and older adults. Boxes represent the 25–75th percentile, with the line indicating the median, and whiskers show the 5–95 percentile. Younger adults had significantly higher antibody secretion rates, \**p* < 0.05; \*\**p* < 0.01, \*\*\**p* < 0.001

**Supplementary Figure 1.** Assay specificity results for pneumococcal antibodies in saliva for IgA, IgM and IgG. A total of 5 saliva samples were tested within the same plate, without manipulation (normal saliva) and following pre-incubation with PPV23, which contains all serotypes quantitated in the multi-plex Luminex assay. Figures illustrate the effects of pre-incubation with PPV23 on median florescent intensity (MFI), averaged across samples, for each specific antibody. Following absorption with antigen, IgA serotypes MFI’s were reduced to a mean level of 16% (range, 6–43%) of the unabsorbed level. All serotypes were reduced to < 20% of the unabsorbed level, with the exception of Pn7F, Pn14 and Pn19A. IgM serotypes MFI’s were reduced to a mean level of 13% (range, 5–28%) of the unabsorbed level following incubation. All serotypes were reduced to < 20% of the unabsorbed level, with the exception of Pn7F and Pn14. IgG serotypes MFI’s were reduced to a mean level of 40% (range, 20–72%) of the unabsorbed level.

**Supplementary Figure 2.** Assay linearity results for pneumococcal antibodies in saliva for IgA, IgM and IgG. Figures illustrate the expected (dashed line) versus the observed (continuous black line) total pneumococcal antibody concentration (all serotypes combined) averaged for 5 different saliva samples. For IgA, at expected concentrations of 50%, 25%, 12.5% and 6.25%, mean observed concentrations (and ranges across serotypes) were 46% (36–55%), 22% (9–29%), 15% (5–31%) and 11% (3–25%), indicating deviations of expected versus observed between -16% to 18% across serotypes and dilutions. The majority of serotypes acquired concentrations maintained within -10 to 10% of the expected concentration across serotypes, except Pn9V, 14 and 18C that were within -20 to 20%. For IgM, at expected concentrations of 50%, 25%, 12.5% and 6.25%, mean observed concentrations (ranges across serotypes) were 54% (34–73%), 24% (5–43%), 16% (1–35%) and 2% (1–3%), equivalent to discrepancies of expected versus observed between -16% to 23% across serotypes and dilutions. For IgG, at expected concentrations of 50%, 25%, 12.5% and 6.25%, mean observed concentrations (range across serotypes) were 35% (26–64%), 10% (7–18%), 7% (3–12%) and 6% (2–12%), indicating deviations of expected versus observed between -24% to 15% across serotypes and dilutions.