Saliva-derived DNA performs well in large-scale, high-density SNP microarray studies

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Saliva derived DNA in genome-wide association studies

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Abstract

As of June 2009, 361 genome-wide association studies (GWAS) had been referenced by the HuGE database(1). GWAS require DNA from many thousands of individuals, relying on suitable DNA collections. We recently performed a Multiple Sclerosis (MS) GWAS where a substantial component of the cases (24 %) had DNA derived from saliva. Genotyping was performed on the Illumina genotyping platform using the Infinium Hap370CNV DUO microarray. Additionally we genotyped ten individuals in duplicate using both saliva and blood derived DNA. The performance of blood versus saliva derived DNA was compared using genotyping call rate, which reflects both the quantity and quality of genotyping per sample, and the ‘GCScore’, an Illumina genotyping quality score, which is a measure of DNA quality. We also compared genotype calls and GCScores for the 10 sample pairs. Call rates were assessed for each sample individually. For the GWAS samples we compared data according to source of DNA and centre of origin. We observed high concordance in genotyping quality and quantity between the paired samples, and minimal loss of quality and quantity of DNA in the saliva samples in the large GWAS sample with the blood samples showing greater variation between centres of origin. This large dataset highlights the usefulness of saliva DNA for genotyping, especially in high-density SNP microarray studies such as GWAS.
Introduction

Genome wide association studies (GWAS) require the collection of thousands of DNA samples to attain sufficient power to map loci responsible for complex diseases. DNA for such studies is usually extracted from lymphocytes, with blood collected from participants by phlebotomy. A recent study of DNA collection in a Danish nurse cohort(2) showed a markedly greater response rate in recruitment of DNA samples from saliva versus blood (72% versus 31% of invited participants returned samples respectively). These differences in response stemmed from a variety of issues including the commitment required to provide blood by attending a clinic and uneasiness, or inability, to undergo phlebotomy. In comparison, saliva collection can be done at home, without professional help, and is much less invasive than phlebotomy. However, saliva-derived DNA is often contaminated with large amounts of bacterial DNA and it is notoriously difficult to determine the relative proportion of human DNA. Moreover, different protocols are required for purification and quantitation of DNA derived from blood and saliva (3, 4). It is therefore entirely plausible that DNA derived from these different sources might show systematic differences in genotyping efficiency and accuracy.

We recently performed a Multiple Sclerosis GWAS with 1618 MS cases and 3413 controls under the auspices of the Australia and New Zealand MS Genetics Consortium (ANZgene) (5). Twenty-four percent of MS cases had genotyping performed on DNA derived from saliva and the remaining samples were derived from blood. The dataset used in the current study consisted of genotypes from these MS cases, some of the controls, as well as some samples that were not included in the published GWAS. In addition a small dataset consisting of 10 sample duplicates,
where both saliva- and blood-derived DNAs were available, was also examined. These data were used to investigate the relative genotyping performance of blood and saliva derived genomic DNA using the Illumina SNP microarray platform.
Materials and Methods

Genomic DNA (gDNA) from Australian MS cases and controls was extracted from whole blood using a variety of standard laboratory approaches including phenol-chloroform extraction(6), salting out(7) and three different commercial kits from Qiagen, Nucleon and Roche, as per manufacturers instructions. gDNA from MS cases from New Zealand (NZ) was isolated from saliva self-collected into Oragene DNA tubes according to the manufacturer's instructions (DNAgenotek). DNA concentrations were assessed using pico green fluorescence, ultraviolet (OD260nm) spectrophotometry and/or on an ethidium bromide–stained low-percentage agarose gel compared to a high-molecular-weight standard. Because of possible bacterial gDNA contamination and difficulty in obtaining reliable pico green and spectrophotometry measurements, all saliva DNA samples were estimated and assessed for their integrity by agarose gel electrophoresis and using at least one other method. Extraction methods, age of samples and time to extraction are summarised by study centre in Supplementary Table 1.

Genotyping was performed on the Illumina genotyping platform with the Infinium Hap370CNV DUO microarrays for both saliva and blood DNAs using the same protocol, at the same facility (Diamantina Institute, Brisbane Queensland), within a six-month time frame.

Each genotype call was associated with a genotyping quality score. For the Illumina platform this is known as a GC Score, or GenCall Score (see Supplementary Material) and ranges from 0 to 1. The GenCall Score is a summary measure consisting of three parts: (i) a SNP specific score known as the GenTrain Score which describes the
clustering properties of the genotyped SNP, (ii) the fit of the current sample to the clustering profile of the SNP, and (iii) the DNAscore which summarises the overall DNA quality of the individual. The GenCall Score thus takes into account SNP properties, sample DNA properties and SNP-specific sample properties. The score has greatest sensitivity in the range of 0.2 to 0.7 with scores >0.7 signalling high quality genotypes. Illumina BeadStudio guideline stipulate that the genotype calling software determines a call to be a “no-call” if the GCScore<0.15, however the GCScores are available for all SNPs, even if the GCScore is below this threshold.

In this analysis we elected to keep all genotyping results regardless of GCScore rather than working with a censored distribution. Thus all SNP markers were used for the call rate and GCScore analyses (N=353,203 out of a total of 370,405 probes on the array), which excludes the copy number variation (CNV) probes (N=17,202) on the array.

The call rate of a sample was determined by the number of SNPs with a genotype call divided by the total number of SNPs considered. Illumina Beadstudio guidelines suggest that samples with a no-call rate> 2% are likely to be of poor quality, so samples which failed this threshold were removed from any further analysis.

**Paired Sample Study**

Venous blood and saliva were collected from 10 consecutively recruited MS patients from study centre Aus9, and the extracted DNAs were genotyped (20 samples in total). The samples were assessed individually for call rate and in a paired comparison for genotype concordance and GCScore similarity.
GWAS Data set

The original recruitment number of ANZgene samples for the MS study was 2000 DNA samples. Of these 75 failed preliminary QC requirements. Of the remaining 1925 samples, 1873 were cases and 52 controls. Stringent QC analysis to remove samples and SNPs using additional methods beyond call rate and GCScore thresholds, pertaining to the published MS GWAS association analysis, led to the inclusion of only 1618 ANZgene cases and 41 controls for the published MS GWAS analysis(5). We chose to use the larger dataset involving 1925 samples for the genotyping quality analysis in the current study because samples and SNPs rejected for other QC reasons than genotyping quality and quantity were still informative for assessment of blood and saliva differences. Control data used in the GWAS dataset (N=3370)(5), which was provided by the Wellcome Trust Case Control Consortium and the Illumina iControl database, were not used in the current study because genotyping quality scores were not available. Case/control status was not taken into account in the analysis.

Ten centres from Australia, designated as Aus1,...,Aus10, contributed blood derived gDNA to the GWAS (Supplementary Table 1). One centre, in New Zealand (NZ), sent out Oragene saliva collection kits within NZ to MS cases self-identified to the investigators through a national prevalence survey. Saliva samples (95% of mailed kits) were returned to the NZ recruitment centre over a 6-month period, and were sent to study centre Aus9 where DNA extractions were performed. All samples were derived from MS patients with ethnic background verbally verified to be Caucasian. All blood and saliva derived DNA samples were genotyped in several genotyping
batches over a six-month period. No randomisation for genotype centres or case/control status was performed over genotype batches due to time constraints. We were unable to investigate genotype batch effects and study centre-specific differences between the samples, such as age of sample, due to strong confounding between genotype batch and study centre.

The GWAS dataset was assessed for genotype call rate across batches, study centres (10 Australian + 1 New Zealand) and DNA source (blood or saliva). The GCScore distribution for each individual was summarised using the three quartiles (25th, 50th and 75th percentile) of the GCScore distribution and these were averaged within relevant pools, either by study centre or by DNA source. The 50th percentile, or second quartile, corresponds to the median. The inter-quartile range (IQR) was taken as the range of values delimited by the values at the 3rd (upper) and 1st (lower) quartiles, respectively. If the data were normally distributed the first and third quartiles would correspond to approximately µ-0.7s and µ+ 0.7s where µ is the mean and s the standard deviation.

The published MS GWAS analysis(5) included an analysis looking for sample stratification that could unduly influence the association analysis. The source of the sample stratification is usually ethnic stratification but can also be potentially caused by technical factors such as genotyping batches or DNA source. The sample stratification analysis was carried out only on the published MS GWAS dataset with the software EIGENSTRAT(8) which identifies data clusters using the statistical technique of principal component analysis (PCA). There is a strong overlap between the set analysed by EIGENSTRAT and the current set of data, as the EIGENSTRAT
analysis included 1659 of the 1925 (86 %) of the samples in the current dataset.
Results

Paired Sample Study
This study allowed a paired comparison of the blood and saliva derived DNA samples, thus removing biological variation and most of the technical variation. The blood DNA sample of one of the paired samples had very poor genotyping quality with a call rate of 0.59. Therefore, this paired sample was dropped entirely from the subsequent statistical analysis leaving nine paired samples. In the remaining nine paired samples there was no significant difference in call rates between blood and saliva DNA for the complete set of SNPs (353,203 SNPs, one sided paired two-sample T-test, $T_8=0.58$, $p=0.3$). We also observed high correlation between GCscores between the samples with a median Spearmann’s rank correlation coefficient of 0.82, range [0.81,0.83], based on all SNPs, regardless of GCscore (Supplementary material, Figures 1-5). There was no difference when SNPs with a GCscore<0.15 were excluded. The number of discordant genotype calls in these paired samples was very low with a median of 0.0035 % discordant genotypes, with range 0.0007 % to 0.0142 %, N=9.

GWAS data analysis
The median SNP call rate (99.82 %, N= 1659) in the GWAS dataset(5) was comparable to other published GWAS, and of the original 2000 MS case DNA samples genotyped, 75 (3.8%) did not pass the call rate threshold of 98%. No saliva-derived samples failed the call-rate threshold. The difference in blood and saliva call rate threshold failures is highly statistically significant ($Z=8.71$, p-value<=2.2e-16),
with saliva derived DNAs much less likely than blood derived DNAs to fail the QC threshold in this study.

The median no-call rate of all samples was 0.0019 (call rate of 99.81%, \( N=1925 \)), for blood DNA was 0.0018 and for saliva DNA it was 0.0024 (Figure 2, Supplementary Data, Table 1). There was a significantly higher rate of no calls in the saliva samples when compared to the blood samples (including only samples with no-call rates <0.02, \( N_{\text{saliva}} = 399 \), \( N_{\text{blood}} = 1526 \), two sample Wilcoxon-rank sum test=171469, p-value<2.2e-16).

Blood derived from three centres (Aus1, Aus3 and Aus10) performed poorly in comparison with blood and saliva derived DNA from other centres (Supplementary Data, Table 2). Aus5 and Aus9 study centre samples also showed additional, intra-batch effects that could not be explained by other factors such as genotyping batch since all samples in these cohorts were either genotyped in the same batch (Aus9) or only two batches (Aus5). Further investigation of these data revealed a relationship with sample ID suggesting a link to time of collection and possibly systematic differences in sample storage and/or processing.

A comparison of GC Scores across studies centres also revealed similar patterns of genotyping bias as evidenced by differences in no-call rate (Supplementary Table 3). The saliva samples had a higher median GC Score than the blood samples, but identical quartiles and IQR. The study centres whose samples had lower call rates (Aus1, Aus3 and Aus10) often had lower IQR and lower median GC Scores indicating that they now represent a biased, or much cleaner, sample. In general the GC Score
quartiles were very similar across all studies centres but this may reflect the non-
discriminatory nature of the GC Score.

The published GWAS data(5) was subjected to stringent QC including principal
components analysis (PCA) using EIGENSTRAT(8) software, but this did not
identify any principal components that clustered with DNA source (data not shown)
suggesting that saliva derived DNA did not have its own SNP genotyping signature in
comparison to blood derived DNA.
Discussion

DNA samples genotyped in our GWAS were collected through 11 different study centres at different times using different extraction methods, and these factors appeared to affect genotype data quality more than the source of DNA, although we were unable to test this hypothesis specifically. The large size of our study permitted averaging over these confounders and a comparison of SNP array-based genotyping performance between blood and saliva derived DNA was performed. Our findings suggest that saliva collected using the Oragene kit provides good quality genomic DNA, which is comparable to blood as a template for SNP genotyping on the Illumina platform. Some studies using different genotyping methods such as PCR for a small number of polymorphisms(9) support our findings, while others have had little success with saliva-derived DNA(10). No other studies have conducted such an extensive comparison of genotyping quality using saliva versus blood derived DNA.

Although both Affymetrix and Illumina claim that Oragene saliva DNA works well on their SNP arrays, there has been no external validation to date. It is plausible that bacterial genomic DNA, which contaminates saliva DNA but not blood DNA, may interfere with genotyping quality. However, our results suggest that the Illumina platform is robust to this potential confounder. This could be due to processing steps and/or the length of the probes on Illumina arrays (50 bp) that may help to overcome the effects of bacterial DNA contamination. If the length of the probes were important one would expect the Affymetrix system to fare worse than Illumina’s because the probes are shorter (25 bp). The Illumina system appears to be quite sensitive to DNA concentration and it is recommended that template DNA be standardised to 50 ng/µl prior to genotyping. Here we standardised both saliva and blood DNA to the
recommended concentration without taking account of the relative proportion of
human versus bacterial DNA in the saliva DNA samples.

Researchers contemplating genetic studies where it is difficult to derive DNA from
blood due to disability or aversion to phlebotomy will be reassured that data generated
from saliva-derived DNA incurs few losses in terms of either genotyping data
quantity or quality in comparison to blood-derived DNA. It is unknown if our
findings will translate to other high throughput genotyping platforms such as
Nimblegen and Affymetrix, although highly variable results were reported for the
500K Affymetrix platform using un-quantitated saliva-derived DNA(4). Interestingly,
DNA from whole genome amplified samples also appear to perform well on the
Illumina platform(11), but genotyping quality and call rate were worse than we
observed for either saliva or blood-derived DNA.

Unlike the Affymetrix platform, the Illumina platform permits multiple samples to be
genotyped per chip, depending on the chip design. Illumina fixed content genome
wide association study SNP chips vary from single sample designs to duo designs,
like the chip used for this study, up to designs which can assay 12 samples
simultaneously on a single chip. This study was not designed to investigate the
influence of array design, and there is as yet no published evidence to indicate that
this is likely to be a significant factor in genotyping quality.

Finally, including all consumables and labour costs each saliva sample used in this
study cost on average USD$80 to collect and process. Here we have shown that saliva
DNA is high quality and suitable as a template for array-based SNP genotyping, at
least for the Illumina Infinium genotyping platform. The Oragen kit also enables self-collection and therefore presents minimal inconvenience to the participant, resulting in high response rates. Further, we have demonstrated that saliva samples can be sent in the mail to a central collection point, thereby reducing transportation costs and the risk of duplication. These financial and logistical benefits will positively impact other genetic studies seeking to expand collections for future research.

COMPETING FINANCIAL INTERESTS

None.

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REFERENCES


FIGURE LEGENDS

Figure 1. No-call rates for the GWAS by study centre

No-call rates across all Australian and New Zealand samples (N=1925), which passed the initial 98%, call rate threshold. Call rates are coloured according to study centre. The New Zealand samples (black) are all saliva derived DNAs. All Australian samples are from blood derived DNA. The black line is the median overall no-call rate (0.0019). Samples are plotted (when possible) according to ID number. Not all study centres have numerical IDs.
Figure 1 No-call rates for the GWAS by study centre
REFERENCES

SUPPLEMENTARY FIGURES

Supplementary Figures 1-5

Scatter plots of GC Scores of the ten blood saliva paired sample comparisons using all SNPs that passed QC. Sample 10 was excluded from the statistical analysis since the blood sample failed QC but is displayed here for comparison.
Supplementary Figure 1 GC Scores for paired saliva blood DNA samples 1 and 2
Supplementary Figure 2 GC Scores for paired saliva blood DNA samples 3 and 4
Supplementary Figure 3 GC Scores for paired saliva blood DNA samples 5 and 6
Supplementary Figure 4 GC Scores for paired saliva blood DNA samples 7 and 8
Supplementary Figure 5 GC-Scores for paired saliva blood DNA samples 9 and 10
SUPPLEMENTARY TABLES

Supplementary Table 1 DNA storage and extraction by study centre
Description of storage time, time to extraction, and DNA extraction method for each study centre.

Supplementary Table 2 Quartiles of no-call rates for study/centres in the MS GWAS.
Quartiles for N=1925 samples that passed genotyping QC (no-call rate <0.02) calculated by study centre and DNA source. All/blood is the summary across all Aus centres. IQR is the Interquartile range and represents the range of no-call rates between the third and first quartiles.

Supplementary Table 3 Mean quartiles and IQR of GCScores by study/centre and DNA source.
Mean quartiles for N=1925 samples that passed genotyping QC (no-call rate <0.02) calculated by study centre and DNA source. All/blood is the summary across all Aus centres. IQR is the Interquartile range and represents the range of GC Scores between the third and first quartiles.
Supplementary Table 1 DNA storage and extraction by study centre

<table>
<thead>
<tr>
<th>Study centre</th>
<th>N (% of Total)</th>
<th>DNA source</th>
<th>Age of blood/saliva</th>
<th>DNA extraction method</th>
<th>Time to extraction</th>
<th>Blood storage method</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZ/saliva</td>
<td>399 (20.7)</td>
<td>Saliva</td>
<td>&lt;1 yr</td>
<td>Oragene</td>
<td>1-2 months</td>
<td>NR*</td>
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<tr>
<td>Aus1/blood</td>
<td>12 (0.6)</td>
<td>Mainly PB</td>
<td>&gt; 4 years</td>
<td>Qiagen, salting out</td>
<td>3-4 years</td>
<td>EDTA</td>
</tr>
<tr>
<td>Aus2/blood</td>
<td>72 (3.7)</td>
<td>PB</td>
<td>&lt;3 years</td>
<td>Salting out</td>
<td>2-3 months</td>
<td>EDTA</td>
</tr>
<tr>
<td>Aus3/blood</td>
<td>126 (6.6)</td>
<td>Mainly PB</td>
<td>&gt; 4 years</td>
<td>Qiagen, salting out</td>
<td>3-4 years</td>
<td>EDTA</td>
</tr>
<tr>
<td>Aus4/blood</td>
<td>31 (1.6)</td>
<td>PB</td>
<td>&lt;1 year</td>
<td>Nucleon</td>
<td>&lt; 1 month</td>
<td>EDTA</td>
</tr>
<tr>
<td>Aus5/blood</td>
<td>326 (16.9)</td>
<td>PB</td>
<td>2-8 years</td>
<td>Salting out</td>
<td>&lt; 1 month</td>
<td>EDTA</td>
</tr>
<tr>
<td>Aus6/blood</td>
<td>115 (6.0)</td>
<td>PB</td>
<td>&lt; 10 years</td>
<td>Nucleon</td>
<td>&lt; 2 years</td>
<td>EDTA</td>
</tr>
<tr>
<td>Aus7/blood</td>
<td>67 (3.5)</td>
<td>PB</td>
<td>4 years</td>
<td>Nucleon</td>
<td>2-4 months</td>
<td>EDTA</td>
</tr>
<tr>
<td>Aus8/blood</td>
<td>125 (6.5)</td>
<td>PB</td>
<td>&lt; 2 years</td>
<td>Roche</td>
<td>&lt; 1 month</td>
<td>EDTA</td>
</tr>
<tr>
<td>Aus9/blood</td>
<td>646 (33.6)</td>
<td>PB</td>
<td>5-7 years</td>
<td>Phenol-Chloroform, Nucleon</td>
<td>&lt; 1 year</td>
<td>EDTA</td>
</tr>
<tr>
<td>Aus10/blood</td>
<td>6 (0.3)</td>
<td>PB</td>
<td>1.5 years</td>
<td>Qiagen</td>
<td>&lt; 1 year</td>
<td>ACD</td>
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</tr>
</tbody>
</table>

* NR = not relevant, ¹ PB = peripheral blood, mainly PB = mainly peripheral blood with some cell lines, ² Qiagen, Roche and Nucleon kits used as per manufacturer’s instructions, ³ EDTA = Ethylenediaminetetraacetic Acid, ACD = acid citrate dextrose
### Supplementary Table 2 Quartiles of no-call rates for study centres in the MS GWAS

<table>
<thead>
<tr>
<th>Study centre</th>
<th>N (% of Total)</th>
<th>Median 1$^\text{st}$ Quartile 3$^\text{rd}$ Quartile IQR</th>
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<tbody>
<tr>
<td>NZ/saliva</td>
<td>399 (21)</td>
<td>0.0024 0.002 0.0032 0.0012</td>
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<tr>
<td>Aus1/blood</td>
<td>12 (0.6)</td>
<td>0.005 0.0047 0.005 0.0003</td>
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<td>Aus2/blood</td>
<td>72 (3.7)</td>
<td>0.0021 0.0018 0.0027 0.0009</td>
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<tr>
<td>Aus3/blood</td>
<td>126 (6.6)</td>
<td>0.0042 0.0034 0.0073 0.0039</td>
</tr>
<tr>
<td>Aus4/blood</td>
<td>31 (1.6)</td>
<td>0.0022 0.0018 0.0029 0.0011</td>
</tr>
<tr>
<td>Aus5/blood</td>
<td>326 (16.9)</td>
<td>0.0018 0.0015 0.0025 0.001</td>
</tr>
<tr>
<td>Aus6/blood</td>
<td>115 (6)</td>
<td>0.0017 0.0015 0.002 0.0005</td>
</tr>
<tr>
<td>Aus7/blood</td>
<td>67 (3.5)</td>
<td>0.0017 0.0015 0.002 0.0005</td>
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<tr>
<td>Aus8/blood</td>
<td>125 (6.5)</td>
<td>0.002 0.0016 0.0023 0.0007</td>
</tr>
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<td>Aus9/blood</td>
<td>646 (33.6)</td>
<td>0.0016 0.0015 0.0018 0.0003</td>
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<td>Aus10/blood</td>
<td>6 (0.3)</td>
<td>0.0042 0.0039 0.0076 0.0037</td>
</tr>
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<td>All blood</td>
<td>1526 (79.3)</td>
<td>0.0018 0.0015 0.0024 0.0009</td>
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<tr>
<td>All</td>
<td>1925 (100)</td>
<td>0.0019 0.0016 0.0026 0.001</td>
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Supplementary Table 3 Mean Quartiles and IQR of GCScores by study centre and DNA source.

<table>
<thead>
<tr>
<th>Study centre</th>
<th>N (% of Total)</th>
<th>Median</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Quartile</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; Quartile</th>
<th>IQR</th>
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<tbody>
<tr>
<td>NZ/saliva</td>
<td>399 (20.7)</td>
<td>0.625</td>
<td>0.037</td>
<td>0.985</td>
<td>0.947</td>
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<tr>
<td>Aus1/blood</td>
<td>12 (0.6)</td>
<td>0.617</td>
<td>0.046</td>
<td>0.984</td>
<td>0.938</td>
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<tr>
<td>Aus2/blood</td>
<td>72 (3.7)</td>
<td>0.625</td>
<td>0.039</td>
<td>0.984</td>
<td>0.945</td>
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<td>Aus3/blood</td>
<td>126 (6.6)</td>
<td>0.625</td>
<td>0.037</td>
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<td>0.945</td>
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<td>Aus4/blood</td>
<td>31 (1.6)</td>
<td>0.615</td>
<td>0.037</td>
<td>0.983</td>
<td>0.946</td>
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<td>Aus5/blood</td>
<td>326 (16.9)</td>
<td>0.618</td>
<td>0.039</td>
<td>0.984</td>
<td>0.945</td>
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<td>0.949</td>
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<td>0.035</td>
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<td>0.949</td>
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<td>Aus8/blood</td>
<td>125 (6.5)</td>
<td>0.616</td>
<td>0.038</td>
<td>0.984</td>
<td>0.946</td>
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<td>Aus9/blood</td>
<td>646 (33.6)</td>
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<td>6 (0.3)</td>
<td>0.621</td>
<td>0.053</td>
<td>0.982</td>
<td>0.930</td>
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<td>All blood</td>
<td>1526 (79.1)</td>
<td>0.619</td>
<td>0.037</td>
<td>0.985</td>
<td>0.947</td>
</tr>
<tr>
<td>All</td>
<td>1925 (100)</td>
<td>0.620</td>
<td>0.036</td>
<td>0.985</td>
<td>0.949</td>
</tr>
</tbody>
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