Determining the validity of hospital laboratory reference intervals for healthy young adults participating in early clinical trials of candidate vaccines

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This was a retrospective study to determine the validity of institutional reference intervals for interpreting biochemistry and hematology results in healthy adults in the context of clinical trials of preventive vaccines. An example population of 974 healthy adults participating in clinical trials at the Jenner Institute, Oxford, UK, between 1999 and 2009 was studied. Methods for calculating the central 95% ranges and determining the coefficients of within person variation were demonstrated. Recommendations have been made as to how these data can be usefully applied to the interpretation of blood results in healthy adult subjects for the purposes of clinical trial inclusion decisions and post-vaccination safety monitoring.

Introduction

The accurate interpretation of biochemical and hematological blood analyses are an important aspect of clinical trials of preventive vaccines, both for trial inclusion decisions and post-intervention safety evaluation. Reference intervals (RIs) are typically derived from historical literature values, laboratory instrument manufacturers or appropriate statistical analysis of routine clinical laboratory patient data, all of which can introduce sampling bias.[1,2] Blood results are affected by individual and laboratory factors, such as differences in age, gender, ethnicity, laboratory methods and laboratory instruments.[3-5] While the gold standard is to minimize laboratory and population differences by computing institutional RIs, there are inherent problems with obtaining a sufficient sample size for such analyses, so validation of externally obtained RIs is recommended.[6] In clinical trials of preventive vaccines, abnormalities in blood results from healthy young adults are usually defined by comparison with all age institutional or published RIs.[7-10] There is a need to validate the RIs used in clinical trials to ensure they are applicable to the trial population. The data set of blood results from vaccine clinical trial programmes in healthy young adults at the Jenner Institute, Oxford, UK, is sufficiently large for calculation of the central

95% ranges for comparison with the institutional RIs. One of the interventions, MVA85A, a candidate tuberculosis vaccine, has been administered to over 100 healthy adults in a series of early clinical trials.[11] Retrospective analyses of blood results obtained from asymptomatic adult subjects at the Jenner Institute and subjects vaccinated with MVA85A were conducted and the literature reviewed in order to identify and account for discrepancies between the study population and the institutional RI. The main aim of these analyses was to develop recommendations for the interpretation of blood results obtained from healthy adult clinical trial participants in Oxford, UK.

Results

Clinical trials, subjects and sample results. Between 1999 and 2009, 38 clinical trials of candidate malaria, tuberculosis, influenza and hepatitis C vaccines in healthy adult subjects were conducted (unpublished trial by Porter et al., NCT00548444).[12-37] In total, 1106 screening appointments were attended by 974 subjects, of which 70% were enrolled in a clinical trial (**Fig. 1**). Subjects aged 18 to 60 y were screened for inclusion in clinical trials, with a median female age of 25.3 y (IQR 9.1). The median age of male subjects was 26.3 y (IQR 9.7). Ethnicity data was not

Screened for clinical trials 1999-2009
subjects n=974 (males n=496, 51%); Screening attendances n=1106

Included in clinical trial n=777 (males n=383, 49%)
Excluded from clinical trial n=325 (males n=175, 54%)
• Specific trial criteria n=106
• Medical assessment n=48
• Abnormal haematology or biochemistry results n=31
• Blood borne virus detected n=3
• Other / not known n=137

Excluded from this analysis: n=158
• *Age over 40 years: n=108*
• *Missing essential data: n=18*
• *Medical assessment not healthy: n=26*
• *Illicit drug use: n=6*

Eligible for inclusion in analysis:
n=818 (males n=410, 50%)

Between-person analysis

1. **Calculation of 95% ranges**
2. **Further evaluation:**
   • **ALT**
   • **Potassium**

Within-person analysis

Co-efficient of variation
Longitudinal data available
n=483 (males n=234, 48%)

Paired analysis of results pre- and post-intervention with candidate tuberculosis vaccine, MVA85A
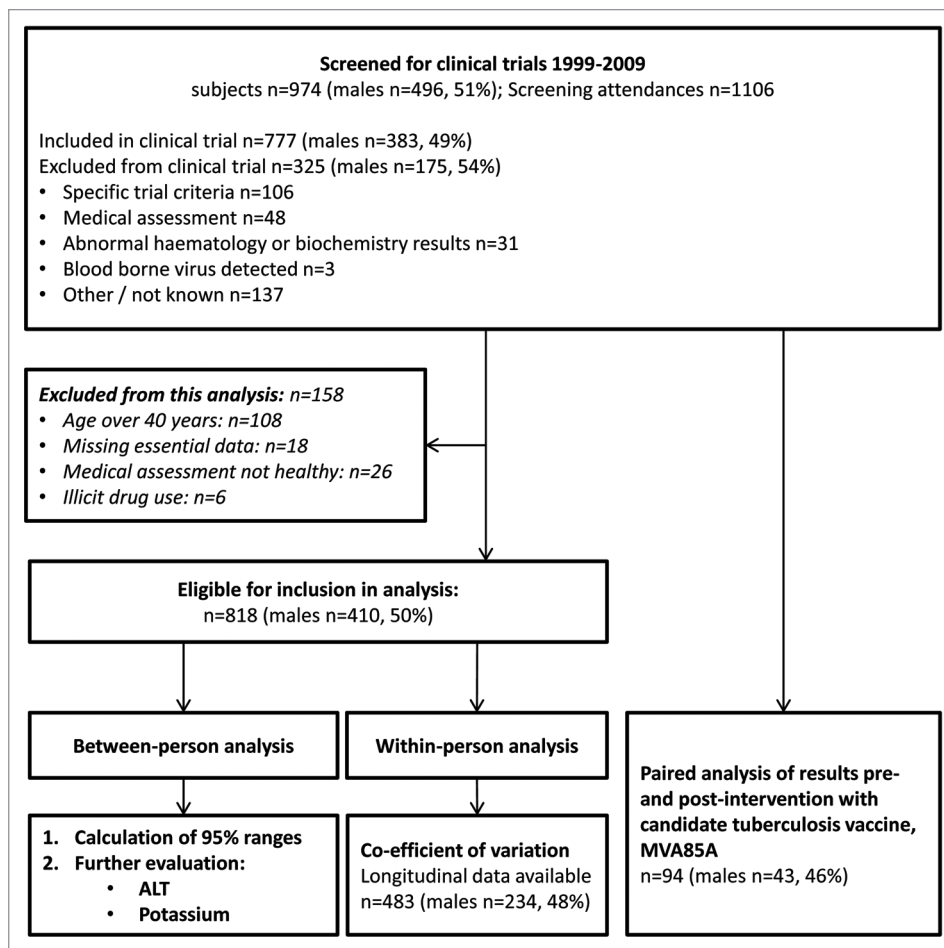n=94 (males n=43, 46%)

Figure 1. Sample selection for analysis. The flow of subject data which was included in each of the four analyses is shown. Where the same subject participated in more than one clinical trial, only the results from the first screening attendance were included in the between-person analyses.

routinely collected for all trials, but in a subset of 115 subjects participating in TB vaccine clinical trials, 80–100% of subjects were born in Europe.[12,13,19,28]

Between-person variation. The number of subjects aged 40 y and under who were included in the between-person analyses was 818, of which 410 (50.1%) were male (**Fig. 1**). Female subjects' median age was 24.7 y (IQR 6.6), and the median age of males analyzed was 25.1 y (IQR 6.7).

Fewer than 20 datapoints from samples run on the Bayer Axon were within each analyte and gender subgroup, which was insufficient for between-instrument comparisons. Samples run on the Bayer Axon were therefore excluded from further analysis. No clinically relevant differences in results between the Abbott Aeroset and Siemens ADVIA 2400 were identified and results by these two different instruments were combined for subsequent analyses (data not shown).

For all analytes, except alanine aminotransferase (ALT) in males, there were no differences in results between age tertiles using Kruskal-Wallis (data not shown). Results for subjects aged 40 y and under were combined for subsequent analyses, except for ALT results in males. The proportions of results outside the

institutional RI were determined, and the calculated 95% ranges for each analyte were compared with the institutional RI (**Table 1**). ALT and potassium results were examined in further detail.

The calculated 95% range for ALT in female subjects was the same as the institutional RI. For male subjects, there were differences in ALT results between age tertiles (Kruskal-Wallis p = 0.0001) and a trend of increasing ALT result with increasing age (nonparametric trend p = 0.001) (**Fig. 2**). Since there were fewer than 120 data points within each age tertile, 95% ranges of ALT results for two age quantiles were calculated. For males aged 18 to 24 y, the calculated 95% ranges were similar to the institutional RI. For males aged 25 to 35 y, 12% of results were above the upper limit and the calculated upper limit was more than 1.5 times greater than the institutional upper limit for ALT. Repeat samples were available in 29 of the 37 (78%) subjects 18 to 40 y with an elevated ALT. Of these subjects, 11 (38%) had repeat samples within the institutional RI limits; 14 (48%) subjects' repeating ALT results were mildly abnormal (up to twice the upper limit); and four (14%) subjects' repeating ALT results were moderately abnormal (more than twice the upper limit).

One quarter of all potassium results were below the lower limit for the institutional RI and the calculated upper and lower limits were lower than those for the institutional RI. Median monthly potassium was lower in the summer than the winter months (**Fig. 3A**) and correlated negatively with mean maximum monthly temperature (Spearman rho = −0.76, p < 0.001). The frequency of below range potassium results was highest in the summer months (**Fig. 3B**).

Within-person variation. Analytes showing the greatest within-person variability were bilirubin, ALT, white cells and urea (**Table 2**). The most stable analytes with least within-person variability were sodium, MCV, hemoglobin and albumin. Trends in within-person variation were comparable to previously published figures from large data sets. Pre-and post-intervention results for 94 subjects who participated in clinical trials of a candidate tuberculosis vaccine, MVA85A, were also analyzed (**Table 2**). The median age of female subjects was 26.8 y (IQR 9.2) and male subjects' median age was 26.5 y (IQR 11.9). The median interval between pre-intervention screening samples and vaccination with MVA85A was 28 d (IQR 44). The median interval between vaccination and post-intervention samples was 7 d

Table 1. Study population blood results and the standard laboratory reference intervals (RI)

Analyte	N	Median result (IQR)	Institutional RI	Number of results outside institutional RI		Calculated 95% range following exclusion of outliers	
				Low (%)	High (%)	2.5	97.5
Albumin g/L							
females	379	45 (4)	35–50	0	15 (4.0)	40	51
males	375	47 (4)	35–50	1 (0.3)	37 (9.9)	41	52
ALP IU/L							
females	370	129 (47)	75–250	7 (1.9)	3 (0.8)	78	216
males	374	159 (50)	95–280	6 (1.6)	2 (0.5)	99	266
ALT IU/L							
females	295	16 (8)	10–45	9 (3.0)	9 (3.0)	10	45
males (18–24 y)	135	19 (10)	10–45	3 (2.2)	4 (3.0)	11	46
males (25–35 y)	135	24 (15)	10–45	3 (2.2)	12 (12.6)	12	71
Bilirubin μmol/L							
females	377	9 (4)	3.0–17.0	4 (1.0)	19 (5.0)	5	18.0
males	374	12 (6)	3.0–17.0	1 (0.3)	58 (15.5)	6	24.5
Creatinine μmol/L							
females	380	80 (13)	70–150	49 (12.9)	0	63	101
males	374	93 (14)	70–150	6 (1.6)	0	74	115
Potassium mmol/L							
females	378	3.6 (0.5)	3.5–5.0	98 (25.9)	6 (1.6)	3	4.3
males	376	3.7 (0.4)	3.5–5.0	90 (23.9)	3 (0.8)	3.2	4.3
Sodium mmol/L							
females	380	139 (2)	135–145	2 (0.5)	2 (0.5)	136	142
males	376	140 (2)	135–145	0	4 (1.1)	137	144
Urea mmol/L							
females	346	4.1 (1.2)	2.5–6.7	9 (2.6)	6 (1.7)	2.4	5.8
males	354	4.9 (1.4)	2.5–6.7	2 (0.6)	22 (6.2)	3.2	7
Hemoglobin g/dL							
females	373	13.2 (1.2)	12.0–15.0	29 (7.8)	10 (2.7)	11.5	14.8
males	377	15 (1.3)	13.0–17.0	7 (1.9)	5 (1.3)	13.2	16.5
MCV fL							
females	373	89.9 (9.9)	83–105	24 (6.4)	1 (0.3)	81.7	97.6
males	377	88.5 (5.2)	83–105	21 (5.6)	0	81.2	97.1
Platelets ×10⁹/L							
females	373	261 (79)	150–400	3 (0.8)	7 (1.9)	173	375
males	377	229 (58)	150–400	18 (4.8)	2 (0.5)	154	359
White cells ×10⁹/L							
females	373	6.2 (2.2)	4.0–11.0	24 (6.4)	10 (2.7)	3.8	10.7
males	377	5.7 (2.0)	4.0–11.0	34 (9.0)	5 (1.3)	3.6	10.2

(IQR 0). One post-intervention sodium result (109 mmol/L) for one subject was excluded from the analysis. This result was deemed aberrant as there were no associated symptoms of hyponatraemia and sodium was within the normal range (139 mmol/L) on a sample taken 2 d later. The repeat sodium result of 139 mmol/L was included. Albumin and bilirubin results were lower post-intervention compared with pre-intervention but no results fell below the lower limit of the institutional RI. Hemoglobin and MCV were transiently reduced one week post-intervention. Hemoglobin fell below the lower limit of the institutional RI one week post-intervention in five (6%) of subjects, all of whom were female, but in all cases returned to normal by 12 weeks post-intervention.

Proposed criteria for interpreting blood results in clinical trials. Background evidence from published literature or
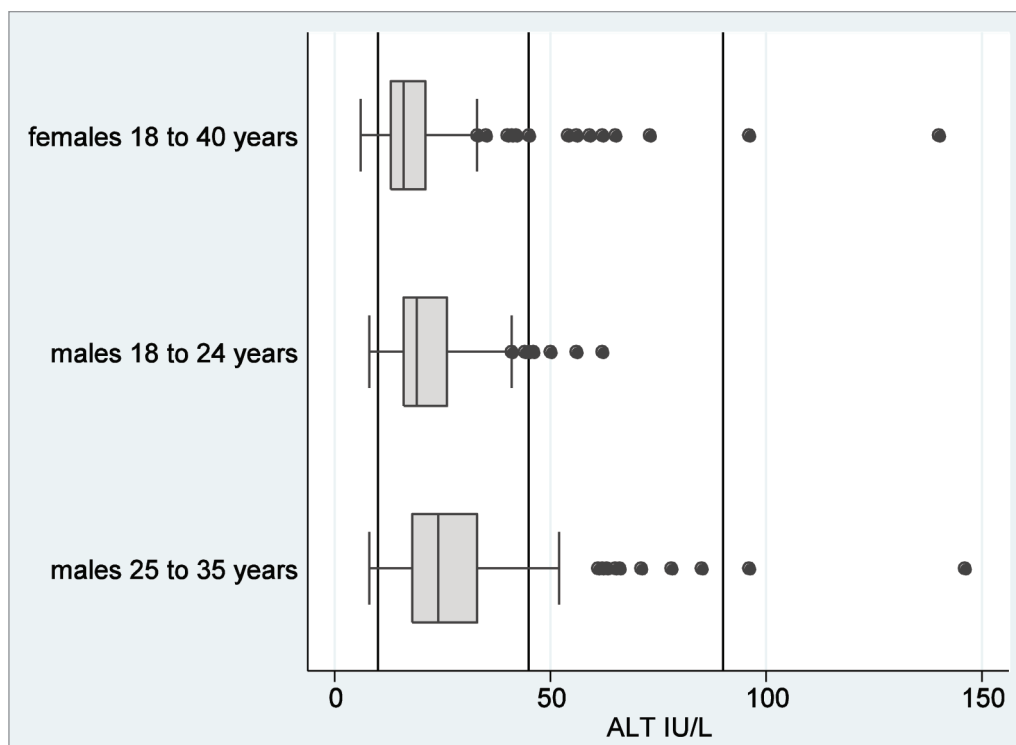
Figure 2. ALT results for females aged 18 to 40 y; males aged 18 to 24 y and males aged 25 to 35 y are shown in a box plot. Boxes contain median lines and whiskers show the interquartile ranges. Dots are individual outliers.

guidelines from recognized sources for each analyte, with relevance for young, asymptomatic adult subjects, are summarized in **Table 3**. Cautionary ranges and expected variation from baseline are shown in **Table 4**.

Discussion

The validity of the institutional RIs for the young adult study population was first evaluated by determining the proportion of results which were outside the range of the institutional RI. The institutional RIs were two sided, defining the upper and lower limits of 95% of results. No analyte had both 2.5% of results above the upper limits and 2.5% of results below the lower limits, as would be expected. For analytes such as albumin and ALP, this may reflect differences between the all adult reference populations, often patients, from which institutional RIs were derived and the population of young, asymptomatic adults volunteering to participate in vaccine clinical trials at the Jenner Institute, Oxford (**Table 3**).

One in four potassium results in the study were below the lower limit of the institutional RI for potassium. Potassium levels in ex-vivo blood samples are not stable when stored prior to analysis unless centrifuged at the point of care.[52] Seasonal pseudohypokalaemia has been described and is hypothesized to be caused by ex-vivo sodium-potassium ATPase-mediated increased uptake of potassium by metabolically active cells in warm conditions.[48,53] We speculate that pseudohypokalaemia due to storage of the samples at environmental temperature (centrally heated building in winter; no air conditioning in summer) prior to transport for

analysis accounted for the downward shift potassium levels compared with the institutional RIs. Centrifuges have been installed in primary care practices, on the basis that correct results can only be ensured by centrifugation of samples in gel separator tubes.[53] In Scotland, source centrifugation of samples taken in primary care facilities successfully reduced the frequency of pseudohyperkalaemia.[54] We propose a prospective study to investigate the effect of centrifugation at source on potassium results.

Discrepancies between the study population and the institutional RIs were further evaluated by using a well-recognized method for defining RIs, which is the basis for current guidelines.[2,6,55,56] The central 95% of results for each analyte, subgrouped by gender, were calculated following removal of outliers. This method aims to maximise sensitivity and specificity by obtaining information from the extremes of the sample, while avoiding outliers adversely influencing the analysis. The importance of the calculated 95% ranges is that there is a danger that a clinically significant result which falls outside the calculated range for this healthy adult population, but within the limits of the institutional RI for all adults, may be clinically significant, but would be overlooked if referring only to the institutional RIs.

The calculated 95% range for ALT in males aged 25 to 40 y was higher than the institutional RI, reflecting increased levels in this subgroup, but adherence to the institutional RI for ALT is essential. Minimally elevated ALT levels are of clinical significance (**Table 3**) and a number of groups have advocated reducing current upper normal limits.[4,57] Current recommendations are for investigation of persistently elevated ALT of any degree above standard upper limits in asymptomatic individuals.[58] We have,
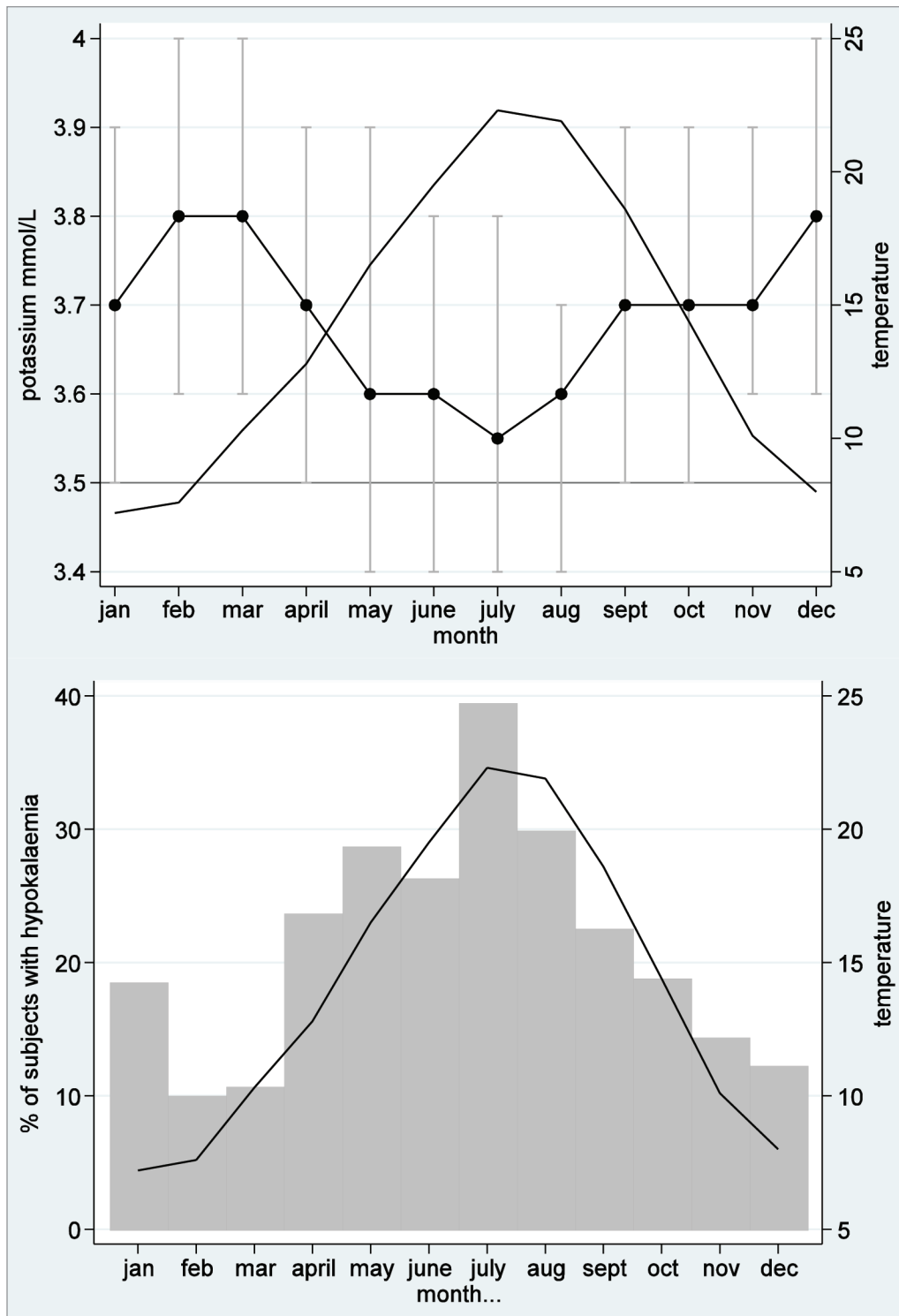
Figure 3. Potassium and environmental temperature. (**A**) Median monthly potassium and mean monthly maximum temperature. Median potassium results for each month are shown as connected points, with capped bars indicating the interquartile ranges. The line represents the mean monthly maximum temperature in Oxford, UK, from 1979–2000. The nadir median monthly potassium results corresponded with the summer months and the peak environmental temperature. A negative association between monthly median potassium and mean temperature was found (Spearman rho = −0.76, p < 0.001). (**B**) Proportion of results indicating hypokalaemia and mean monthly temperature. The vertical bars indicate the percentage of samples with potassium results below the lower limit of the RI (3.5 mmol/L) each month. The line connects the mean maximum temperature for each month. Low potassium results were more frequent in the summer months than the winter months.

Table 2. Within-person variation and comparison of results pre-and post-MVA85A vaccination

Analyte	N	Males (%)	CVwp[a] for study population	Published CVwp[38]	Pre-intervention results median (IQR)	Post-intervention results median (IQR)	Median difference (IQR)	p value[b]
Albumin	476	229 (48)	5.0%	3.1%	46 (4)	45 (4)	1 (4)	< 0.001
ALP	470	228 (49)	11.0%	6.4%	138 (57)	139 (60)	3 (21)	0.45
ALT	339	160 (47)	23.8%	18.0%	20 (10)	18 (10)	1 (8)	0.70
Bilirubin	479	231 (48)	28.4%	23.8%	10 (4)	10 (6)	1 (5)	0.01
Creatinine	481	231 (48)	7.8%	6.0%	88 (16)	88 (22)	0 (10)	0.47
Potassium	481	230 (48)	9.1%	4.8%	3.7 (0.5)	3.7 (0.3)	0 (0.4)	0.75
Sodium	449	231 (50)	1.3%	0.7%	139 (2)	139 (2.5)	0 (3)	0.44
Urea	339	223 (48)	16.2%	12.3%	4.3 (1.2)	4.4 (1.4)	−0.1 (1.2)	0.39
Hemoglobin	483	234 (48)	4.1%	2.8%	14.2 (2.3)	13.8 (2.2)	0.4 (0.8)	< 0.001
MCV	483	234 (48)	2.3%	1.3%	89.1 (5.4)	89.2 (5.4)	0.3 (2.2)	0.01
Platelets	483	234 (48)	10.0%	9.1%	260 (81)	256 (85)	−4 (38)	0.09
White cells	483	234 (48)	19.6%	10.9%	5.9 (2.2)	5.7 (2.1)	0.1 (−1.6)	0.18

[a]CVwp, within person coefficient of variation; [b]Wilcoxon Signed Rank paired analysis.

therefore, proposed a clinical algorithm for the management of elevated ALT results in clinical trial populations (**Fig. 4**).

Understanding the expected degree of within-person variation for an analyte and the factors associated with variations is of particular importance for the interpretation of sequential results. Consistent with previous published data, bilirubin and ALT showed the greatest within-person variation. Transient fluctuations are common and may be caused by recent alcohol consumption, viral illnesses, concomitant medications and exercise patterns.[59] For these analytes, transient fluctuations need to be distinguished from persistent elevations, by repeat testing and possible causes of transient fluctuations documented, by relevant history taking. It is intuitive to expect that analytes which vary greatly are likely to be the most frequently reported as abnormal in post-vaccination safety evaluation. For example, in a recent study of two candidate tuberculosis vaccines, MVA85A and FP85A, post-MVA85A vaccination ALT was elevated in one subject.[12] The paired analysis of 94 pre- and post-MVA85A vaccination blood results, was reassuring in showing no frequent effect of MVA85A vaccination on ALT results.

The paired pre- and post-intervention analysis identified a transient reduction in hemoglobin and MCV in females, coinciding with the time in the trials when the greatest blood volumes were drawn. When the outcome measures of a clinical trial include the evaluation of vaccination induced immune responses, sizable blood samples (60–80 ml) are required from each participant at regular intervals. For trials such as these, we recommend adherence to national transfusion guidelines for the selection of blood donors, when setting trial inclusion criteria. In the UK, the minimum Hemoglobin levels for blood donors are 12.5 and 13.5 g/dl females and males, respectively.[60]

The strengths of this study include the use of healthy young adult subjects, all of whom had a medical assessment. Subjects with pre-existing medical problems, alcohol excess or infection with blood borne viruses were excluded from the analysis. There were over 300 subjects per group, which was above the minimum recommended subgroup size (120 subjects) for non-parametric 95% range calculations.[6] Data used for between-person analyses were taken pre-intervention, i.e., subjects were maintaining their usual lifestyle. Most specimens were taken in the mornings with subjects at rest thereby removing diurnal variation and exertion induced changes as confounding pre-analytical factors. Paired samples for the same individuals were available and all samples were processed in the same laboratory.

Limitations were that the study was retrospective and single centered. Various environmental factors were not controlled. Recreational drug use was an exclusion criterion, but subjects taking certain prescription drugs (such as the oral contraceptive pill) or over-the-counter remedies (e.g., for coryzal and musculoskeletal symptoms) were not excluded. Subjects were not required to fast overnight prior to phlebotomy and recent exercise may have been taken. After phlebotomy, times to sample analysis were not controlled. At the point of laboratory analysis, samples were not frozen, so between-run variation could not be eliminated and replicate analyses of specimens were not performed. The trends in within-person variability were comparable to those previously published; suggesting factors which may have affected results remained consistent between sequential samples for individual subjects. Follow-up data were not available for subjects with significantly or persistently abnormal results who were excluded from participation and referred for primary care follow up. Ethnicity and socio-economic data, anthropometric measurements, iron studies in females and detailed smoking, alcohol, recent diet, exercise and menstrual history would strengthen the study.[61,62]

Taking the results of the analyses and the limitations of the study into consideration, recommendations for the interpretation of blood results in healthy adult subjects have been proposed (**Table 4**). Clearly, the calculated 95% ranges and coefficients of within-person variation are only valid for young, asymptomatic adults whose blood samples are analyzed at the Oxford University Hospitals NHS Trust laboratories, UK. However, the methods described are straightforward and could be applied to other data sets from healthy adult subjects. Key components of

Table 3. Summary of available literature

Analyte	Considerations for young, asymptomatic adults
Albumin	Albumin levels decrease with age.[39] Young, healthy adults are likely to have higher albumin levels due to good nutritional status and liver synthetic capacity.
ALP	ALP levels are stable in young adults, following cessation of growth and bone turnover and increase in the fourth decade.[2,40]
ALT	BMI has the strongest independent association with ALT in healthy populations, but increased ALT levels are also associated with male gender age, peaking at 55 y of age.[4,41,42] Non-alcoholic fatty liver disease (NAFLD) is an important cause of persistently elevated ALT levels in asymptomatic adults and is estimated to have a prevalence of 20–30% in countries such as the UK.[43] ALT levels at the upper end of the normal range are associated with an increased mortality from liver disease.[44]
Bilirubin	The most likely cause of persistently elevated bilirubin in asymptomatic individuals with normal unconjugated bilirubin levels and otherwise normal liver function tests is Gilbert's syndrome, which has a prevalence of 5–10%.[45]
Creatinine	Creatinine production is determined by muscle mass and dietary intake and is affected by gender and ethnicity, with high inter-person variability, so a single RI is not entirely appropriate for a population.[46] Estimates of Glomerular Filtration Rate (eGFR) may take age, gender, ethnicity and body mass into account, but have usually been developed for patients with chronic kidney disease and may not be applicable to an asymptomatic population.[46,47]
Potassium	Potassium has a widely used reference range of 3.5–5.0 mmol/L.[9,10] Ambient temperature is an important cause of spurious hypo- and hyperkalaemia when samples are stored prior to analysis.[48,49]
Sodium	Sodium has a narrow homeostatic range and shows very little inter-person variation.[3] Exercise-associated hyponatraemia occurs after excessive exercise, such as marathons.
Urea	Between-person urea levels are highly variable and are related to protein intake, being higher in males than females and increase with age.[50] Within-person variations are associated with dehydration, alcohol intake and concomitant medication.
Hemoglobin	Hemoglobin RIs are consistently lower in females than males.[9,10]
MCV	Causes of raised MCV include excessive alcohol intake and macrocytic anemia. Iron deficiency and thalassaemia should be considered for low MCV values.
Platelets	Platelet counts are lower in African and Afro-Caribbeans than Caucasians.[5] The most widely used lower limit is 150×10^9/L.[51] Blood smear to exclude pseudothrombocytopenia is indicated for results below the lower limit.[51]
White cells	White cell counts are lower in African and Afro-Caribbeans than Caucasians and are higher in women than men.[5]

the clinical assessment conducting during clinical trial screening, pertinent to the interpretation of blood results, include ethnic origin; detailed alcohol, smoking, diet, exercise and medication history; recent mild illness; history of significant acute or chronic disease; height, weight and blood pressure measurements and urinalysis for blood, protein and glucose. When taking post-intervention blood tests for safety evaluation, concurrent history of recent symptoms, diet, exercise, medication and alcohol intake are recommended.

There is a recognized need to standardise safety reporting across clinical trials of vaccines and the Brighton Collaboration, a global research network, is developing case definitions and guidelines to this end.[63] This study provides important data on which to base further work to validate RIs for young, asymptomatic adults to enable the accurate interpretation of blood results in clinical trials.

Materials and Methods

Subject data set. A retrospective analysis of screening attendances, screening outcomes and laboratory results included all clinical trials recruiting healthy adult subjects at the Jenner Institute, Oxford, UK, between 1999 and 2009. Trials were conducted in accordance with the Declaration of Helsinki and ethical approval obtained. Informed consent was obtained before conducting any study procedures. Subjects were assessed by medical history, physical examination, laboratory assessment and corroborative medical history from General Practitioners, to exclude undisclosed medical conditions. Data from paper case report forms were entered onto an electronic database (Microsoft Excel). Accuracy of electronic data entry was verified by double data entry and crosschecking against paper records.

Laboratory results data set. Non-fasting phlebotomy was performed at the Centre for Clinical Vaccinology and Tropical Medicine, Oxford, during clinical trial clinics (07:30–11:00 a.m.). Biochemistry samples were taken in lithium heparin and hematology samples in potassium EDTA (EDTA) using the Beckton Dickinson (BD) vacutainer collection system. Laboratory specimens were collected within two hours of phlebotomy and transported two miles to the Oxford University Hospital NHS Trust Laboratories for same day analysis. Each blood tube was labeled with a unique study-specific identification (ID) number and laboratory reports were generated for these study IDs. Trial and subject-specific ID numbers were used to retrieve laboratory results electronically.

All methods and instrumentation used were commonly used standardised methods. Albumin (bromocresol green method), alkaline phosphatase (ALP, DEA buffered enzymatic), alanine aminotransferase (ALT, enzymatic method), bilirubin (vanadate oxidation method), creatinine (Jaffe method), sodium and potassium (both ion selective electrode methods) and urea (urease method) were undertaken on three different clinical chemistry analysers over the period studied. Samples analyzed before February 2000 utilized the Bayer Axon analyzer (Bayer Diagnostics); samples from March 2000 and March 2006 were analyzed on an Abbott Aeroset (Abbott diagnostics); and samples

Table 4. Recommendations

Analyte	Institutional RI	Cautionary range[a]	Expected variation from baseline[b]
Albumin g/L			5%
females	35–50	35–39	2–3
males	35–50	35–40	2–3
ALP IU/L			11%
females	75–250	217–250	8–28
males	95–280	267–280	10–31
ALT IU/L			24%
females and males	10–45		2–11
Bilirubin µmol/L			28%
females and males	3.0–17.0		1–5
Creatinine µmol/L			8%
females	70–150	102–150	6–12
males	70–150	116–150	6–12
Potassium mmol/L			9%
females and males	3.5–5.0	4.4–5.0	≤ 0.5
Sodium mmol/L			1%
females and males	135–145		1–2
Urea mmol/L			16%
females	2.5–6.7	5.8–6.7	≤ 1
males	2.5–6.7		≤ 1
Hemoglobin g/dl			4%
females	12.0–15.0		< 1
males	13.0–17.0		< 1
MCV fL			2%
females and males	83–105	98–105	2
Platelets ×10⁹/L			10%
females	150–400	150–172, 376–400	15–40
males	150–400	360–400	15–40
White cells ×10⁹/L			20%
females	4.0–11.0		1–2
males	4.0–11.0		1–2

[a]The cautionary ranges were based upon discrepancies between the institutional RIs and the calculated 95% ranges. [b]The expected variations from baseline were based upon the calculated within person coefficents of variation.

obtained from April 2006 to December 2009 were analyzed on a Siemens ADVIA 2400 (Siemens Diagnostics). RIs remained consistent despite these two changes of instrument as no clinically significant shifts in results were detected by comparison studies (T. James, personal correspondence). Haematological parameters were analyzed on the Sysmex XE-2100 hematology analyzer. The longitudinal stability of all assays was controlled through internal quality control and external quality assurance participation.

Laboratory data were excluded pre-analysis if medical assessment had revealed significant past medical or psychiatric history;

illicit drug use; excessive alcohol intake or if infection with a blood borne virus was detected at screening.

Statistical analysis. Data were analyzed using Stata (Stata statistical software version 9.0, 2005, StataCorp, USA). Data were first visually reviewed to check for heavy skewing by individual results, using histograms and box plots. Continuous variables were not normally distributed and were summarized using median and interquartile range (IQR).

For between-person analyses, results for each analyte were filtered to include one pre-intervention result per subject and were sub-grouped by gender. Differences in the distribution of data between instrument subgroups were compared using Kolmogorov-Smirnov, before combining the data derived from different instruments. Subjects over 40 y of age were excluded and subjects 40 y and under were partitioned into age tertiles. Differences between age tertiles were evaluated by Kruskal-Wallis. Where differences were detected, a non-parametric test for trend across ordered groups was performed. Where no differences between age tertiles were detected, 95% ranges for combined data from subjects of all ages 40 y and under were calculated.

The 95% range for each analyte was calculated using a standard method.[2,6,55,56] Normality of data within each subgroup was assessed graphically by normal probability plots (for evaluating central normality) and normal quantile plots (for identifying deviations from normality at the tails of the data). The Shapiro–Wilk test for skewness and kurtosis and the Kolmogorov-Smirnov test against an empirical normal distribution were performed. Where data were not normally distributed, Box–Cox transformations within each group or subgroup were performed. The effect of normalizing transformation was assessed graphically and statistically as above. The upper (Q1) and lower (Q3) quartiles and IQR (Q1–Q3) of the transformed data were calculated. Data points lying outside the range (Q1–1.5 × IQR) to (Q3 + 1.5 × IQR) were identified as outliers and excluded.[2] Non-parametric 95% ranges were derived by obtaining the 2.5th and 97.5th percentiles of the raw data after exclusion of outliers. Parametric 95% ranges were derived by calculating mean ± 1.96 × standard deviations (SDs) of the raw data after exclusion of outliers.

The analysis of within-person variation included all clinical trial participants 40 y and under for whom at least two sequential results per analyte were available. If a subject had participated in more than one clinical trial, samples were taken from the first clinical trial in which they had participated. The first (r1) and last (r2) retrieved results for each subject were included in the analysis. This minimised any effect of intervention on the analysis, since the first samples for each subject were pre-intervention and the last samples were taken at least 12 weeks post-intervention. The within-person coefficient of variation was calculated using a described method.[62] The square of the coefficient of variation (CV^2) for each subject was calculated by [(within subject variance)/(mean)^2], where within subject variance was [$(r1-r2)^2/2$] and mean result was [(r1+r2)/2]. The within-person coefficient of variation for the sample was obtained by calculating the root mean square; {[√(mean

$CV^2)]*100\}$. The standard error was calculated by [(standard deviation of CV^2s)/($\sqrt{}$sample size)].

The seasonality of potassium results were evaluated by comparing median monthly potassium results against average monthly temperatures. Potassium results from subjects aged 40 y or under were analyzed. Data were grouped by the month the sample was obtained. Monthly average temperatures in Oxford over a 30-y period from 1971–2001 were obtained from the Meterological Office.[64] The association between median monthly potassium and mean monthly temperature was evaluated by Spearman's rho correlation coefficient.

Paired pre- and post-intervention results were analyzed for the candidate tuberculosis vaccine MVA85A (ClinicalTrials.gov registration number of unpublished trial by Porter et al., NCT00548444).[12,13,19,25,28,35] The latest pre-intervention and earliest post-intervention results available were included in the analysis. Results were excluded if samples were obtained greater than one month (31 d) post-intervention, since vaccine-related adverse events and cellular immune responses peak one to two weeks post-vaccination.[21,29,35,65]
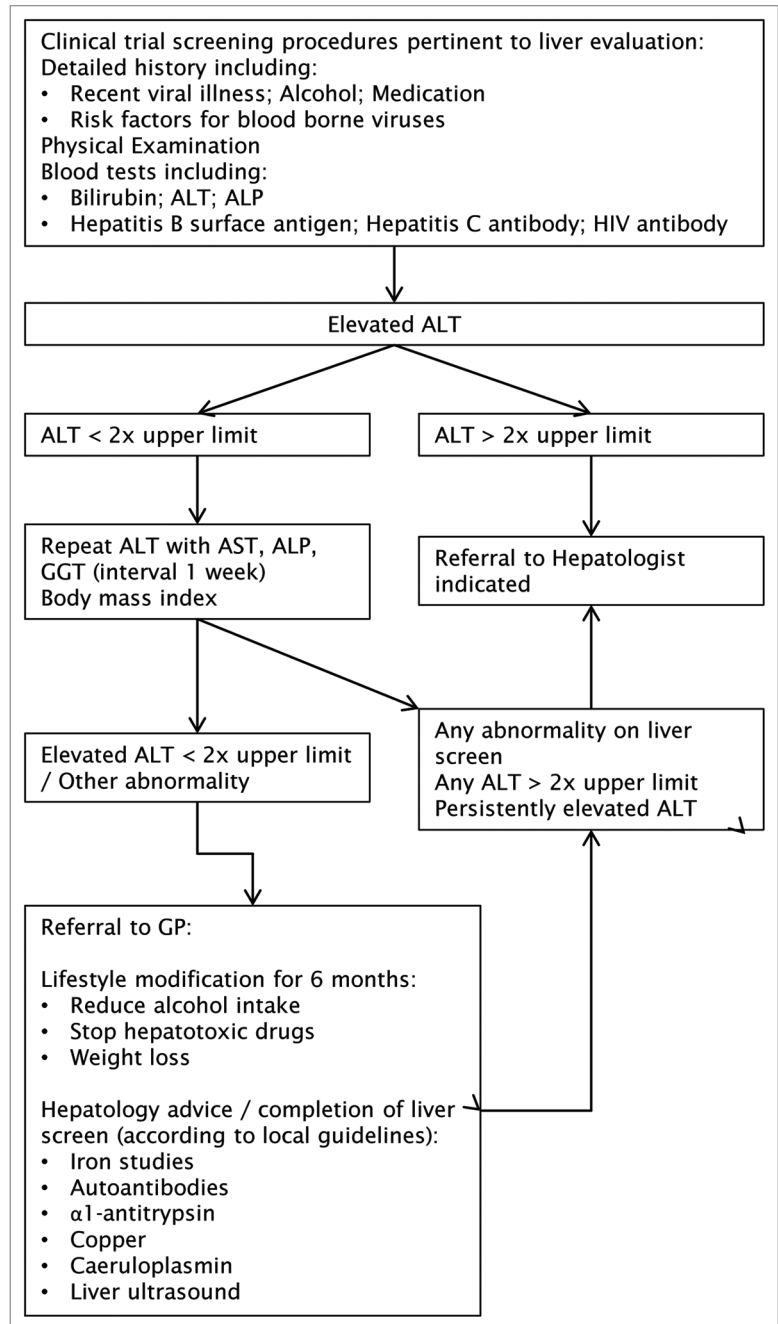
Figure 4. Algorithm for management of elevated ALT in the clinical trial setting.

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