

Interpretation of low reactivity in the Abbott Architect rHTLV I/II assay

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SUMMARY

Objective: The objective of this study is to reduce donor tissue wastage.

Aim: The aim of this study is to determine, in the case of the Abbott Architect rHTLV I/II assay, whether a signal/cut-off (S/CO) ratio higher than the manufacturer's recommendation of 1.0 could be applied to diagnose significant HTLV-1 seroreactivity.

Background: The detection of human T cell leukaemia virus type 1 (HTLV-1) infection is primarily based on serology often utilising random access platforms. Although current assays have high sensitivity and specificity, in low-prevalence regions, significant numbers of false-positive reactions occur. A comprehensive follow-up is difficult within the time frame of organ donation. This can lead to donor tissue wastage.

Methods: A retrospective analysis of 12 250 samples previously tested on the Abbott Architect rHTLV I/II platform and further tested by confirmatory serology/molecular detection to determine the sensitivity and positive predictive value in the S/CO ratio range was conducted.

Results: Where the sample S/CO ratio was >20 (n = 498), HTLV infection was confirmed in all but eight subjects. All of these eight had indeterminate confirmatory results, and none were found to be uninfected. Conversely, in the samples within the S/CO ratio range 1–4 (n = 271), no subject was subsequently found to be HTLV-infected although HTLV infection could not be excluded in all cases, primarily due to lack of follow-up samples (n = 60/271).

Conclusions: Samples with an S/CO ratio of <4.0 on the Abbott Architect rHTLV I/II platform represent a low risk of HTLV infection in the UK, and organs from such donors might

reasonably be considered for transplantation, within the context of appropriate risk–benefit assessment.

Key words: confirmation, HTLV-1, screening, serology, transplantation.

BACKGROUND

Human T-cell leukaemia virus type-1 and type-2 (HTLV-1 and HTLV-2) are members of the genus of deltaretroviruses of the Orthoretrovirinae subfamily of the family of Retroviridae. Discovered in 1980 (Poiesz *et al.*, 1980), HTLV-1 is the cause of adult T cell leukaemia/lymphoma (ATLL), an aggressive malignancy of T cells (Uchiyama *et al.*, 1977; Yoshida *et al.*, 1982); a range of HTLV-associated inflammatory conditions characterised by lymphocytic infiltration of the diseased tissue [reviewed by (Martin *et al.*, 2014)], including HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gessain *et al.*, 1985; Osame *et al.*, 1986); and impaired immunity to several infections resulting in epidemiological and pathological changes with the association between HTLV-1 infection and disseminated *Strongyloides stercoralis* infection (strongyloidiasis) the most striking (Nakada *et al.*, 1987). The combined lifetime risk of HTLV-1-associated disease in carriers approaches 10%. Disease associations with HTLV-2 are less clear but include rare cases of myelopathy and increased prevalence of urinary and respiratory tract infections (Murphy *et al.*, 1997a, 1997b). Evidence of HTLV-2 infection is also detected by HTLV-1 screening assays as there is considerable antibody cross-reactivity. HTLV-1 (and HTLV-2) is transmitted from mother to child, predominantly through breastfeeding; between sexual partners, through unprotected sexual intercourse; through the sharing/reuse of injection equipment; and through the transfusion or transplantation of blood and tissue. HTLV-1-associated myelopathy has been reported within months of transplantation-acquired HTLV-1 infection (Ramanan *et al.*, 2014; Nagamine *et al.*, 2015), leading to calls for increased organ donor screening (Gallo *et al.*, 2016). The original European Union Directive is for 'donors originating from high incidence areas, or with sexual partners originating from those areas or where the donor's parents originate from

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those areas' to have HTLV antibody testing (European Parliament, 2006). In the UK, a low-prevalence region, the advisory committee on the Safety of Blood, Tissues and Organs (SABTO) have indicated that all donors should be screened for HTLV-1/-2 infection (Advisory Committee on the Safety of Blood Tissues and Organs, 2011).

The diagnosis of HTLV-1 (and HTLV-2) is primarily through the detection of HTLV-specific antibodies. Although from the outset, the sensitivity of serology has been high, specificity has been more problematic, particularly with the earliest assays (The HTLV European Research Network, 1996). The need for high specificity has greater significance where screening is conducted within low-prevalence populations, such as blood and tissue donors in non-endemic regions. In the UK, where the prevalence of HTLV infections among first-time blood donors is 5/100000 (Brennan *et al.*, 1993; Davison *et al.*, 2009; NHS Blood and Transplant/Public Health England Epidemiology Unit, 2016), all blood and tissue donors are screened for HTLV infections and donations not used if HTLV reactivity is found on repeat testing. Samples that have reacted in a screening assay are retested and repeat reactive samples subjected to confirmatory tests with Western-blotting-based assays containing discrete gag (p19 and p24) proteins as well as env glycoproteins gp21 and gp46. The use of recombinant env glycoproteins increases the sensitivity of the assay, and antibody reactivity against the recombinant versions of gp46-1 and gp46-2 allows discrimination between the two viruses. In the absence of any bands, the interpretation is HTLV-1 and HTLV-2 negative; the presence of reactivity to multiple gag and env proteins allows the confirmation of HTLV-1 or HTLV-2 infection. In cases where the necessary reactivity to diagnose infection is present but is absent against either HTLV-1 or HTLV-2 rgp46-type-specific glycoprotein or is present against both, an 'untyped' HTLV infection is diagnosed. The presence of bands insufficient to diagnose infection results in an 'indeterminate' interpretation. Confirmation of HTLV reactivity can also be by DNA polymerase chain reaction (PCR) if whole blood is available. However, both confirmatory methods are costly and a time-consuming process that can delay the use of donated blood or tissue and in the worst case result in uninfected tissues being discarded. This potentially has life-threatening consequences for those awaiting vital organs.

One screening assay used in the UK is the Abbott Architect rHTLV-I/II, a chemiluminescent microparticle immunoassay that is approved for the qualitative detection of HTLV-1 and HTLV-2 antibodies in the European Union. The manufacturers specify the interpretation of reactivity, which in the case of the Abbott Architect rHTLV-I/II is any signal-to-cut-off (S/CO) ratio of 1 or above. According to the product insert (accessed 01/03/2017), the sensitivity of the rHTLV-I/II is 100% [95% confidence interval 99.1–100%], whereas the specificity among blood donors is 99.95% (95% CI 99.84–99.9%). Thus, in a population where the prevalence of HTLV-1 infection is 0.005%, among 100 000 donors, 55 reactive results might be anticipated, of which 5 will be due to HTLV-1 infection and 50 will be due to nonspecific reactivity. To determine whether an S/CO

ratio higher than 1 might be used for the discrimination of nonspecific reactivity from infection, a retrospective analysis of the final result (HTLV status) of all samples tested by a national reference laboratory using the Abbott Architect rHTLV-I/II as the first screening test was conducted.

METHODS

During a 5-year period (February 2009 to February 2014), 12 250 blood serum/plasma samples from 10 052 individuals were referred to the Virus Reference Department, Public Health England, for serological diagnosis of HTLV infection. These samples were from all parts of England and from a variety of clinical settings which included the following: samples previously found reactive and referred for confirmatory tests; samples previously untested from patients deemed at risk of HTLV infection; and samples from low-risk patients, such as organ and milk donors. All samples were tested in the Abbott Architect rHTLV-I/II assay (Abbott Laboratories Weisbaden, Germany) according to manufacturer's instructions. Any samples that gave an S/CO ratio of ≥ 1.0 were further tested by Western blot (MP Diagnostics HTLV blot 2.4 Singapore). When unseparated whole blood on ethylenediaminetetraacetic acid (EDTA) was available, Abbott Architect reactive samples were further tested by a nested HTLV DNA PCR (Vandamme *et al.*, 1997; Tosswill *et al.*, 1998). A report was issued to the referring laboratory based on the results of these tests, and a follow-up sample of the whole blood on EDTA was requested for confirmatory testing.

Results were collated and stratified by the S/CO ratio of the first sample received into four strata: 1–4, 4.01–20, 20.01–100 and >100 . These results were then analysed alongside the clinical information. A final HTLV status was allocated to the first sample received from each patient, taking into account any results obtained from follow-up samples. Using the Western blot (WB) and PCR results, samples were given the following status: HTLV-I positive, HTLV-II positive, HTLV untyped, HTLV indeterminate (unresolved) and HTLV negative. The positive predictive value (PPV) of the initial Abbott Architect S/CO ratio results was calculated for each stratum. The values are based on the final result after follow-up.

RESULTS

Of the 10 052 patients tested, 9188 had a negative result on Abbott Architect (i.e. S/CO ratio = <1.0). The remaining 864 patients (11.6%) gave an initial reactive result, and these samples were tested by Western blotting. In addition, follow-up samples were received from 307 of these 864 patients. No individual whose initial sample was reactive with an S/CO ratio >20 was subsequently found to be HTLV-uninfected. In 99%, HTLV infection was confirmed, whereas in the remaining 1%, the final interpretation of the serology was 'HTLV indeterminate'. None were given a final report of HTLV negative (Fig. 1). Where the S/CO ratio in the initial sample was in the range 4–20, a high

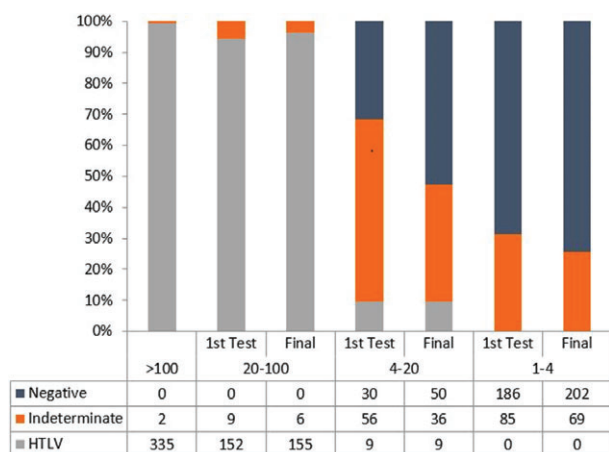


Fig. 1. HTLV-1/-2 final diagnosis of the status of infection according to signal/cut-off ratios from the Abbott Architect rHTLV-I/II.

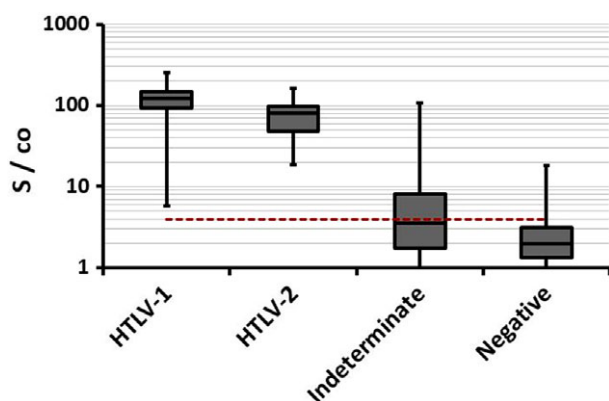


Fig. 2. Signal/cut-off (S/CO) ratios from the Abbott Architect rHTLV-I/II according to the final diagnosis of HTLV-1/-2 infection. Broken line indicates the S/CO ratio of 4. [Colour figure can be viewed at wileyonlinelibrary.com]

proportion of subjects had HTLV-indeterminate results even after testing of follow-up samples. Only 9% of these subjects were confirmed to have HTLV infection, whereas HTLV infection was excluded in 50%. HTLV infection was not confirmed in any individual for whom the initial sample S/CO ratio was <4 (Fig. 1). The spread of S/CO values according to each final diagnosis are presented in Fig. 2.

For S/CO ratios of >100, both the sensitivity and PPV of the initial Abbott Architect result were 100% (95% CI 98.9–100%), respectively. In the range 20–100, the sensitivity and PPV were also 100% (95% CI 97.65–100%), respectively. For S/CO ratios in the range 4–20, the sensitivity remained high, i.e. 100% (95% CI 63.37–100%), but the PPV was only 15.25% (95% CI 7.2–27%). Finally, for the 1–4 S/CO group, the PPV was 0% (95% CI 0–1.8%). Since none of the patients giving an initial S/CO ratio in the range 1–4 were found to be HTLV positive, a more detailed analysis of this group was undertaken.

Samples with an S/CO ratio 1–4

Of 271, 186 (69%) were negative on WB (mean S/CO ratio 1.9, range 1.0–3.95) (Fig. 3). Fifty-one of 186 had a follow-up sample tested: 48 remained WB negative, whereas 3 gave a single band against p24. Whole blood on EDTA was available for two of these p24+ samples, and both were HTLV-1 and HTLV-2 DNA PCR negative. Eighty-five samples were indeterminate by Western blot (mean S/CO ratio 2.09, range 1.01–3.92), and of these, follow-up samples were received from 25 patients. Sixteen of 25 were found to be WB negative and nine remained WB indeterminate. Seven of nine gave only one band on WB, and of these five of five were HTLV DNA negative by PCR. Two had more than one band on WB, and both were PCR negative. No follow-up samples were received from the remaining 60 patients with an indeterminate WB. Of these, the number of bands on WB was as follows: one band = 47 (mean S/CO ratio 1.98), two bands = 8, three bands = 4 and four bands = 1 with no correlation between S/CO ratio and band pattern (the mean S/CO ratio for samples with more than one band on WB was 2.1).

DISCUSSION

The goal of screening is to make blood and tissue transplantation as safe as possible. During the preparation of this manuscript, the authors were involved in a number of cases where low titre reactivity was observed in potential donors, both stem cell and solid organ, which in a number of cases resulted in organs not being transplanted. Subsequent to the decision to discard the organs, further investigations confirmed that the donors were uninfected. In other cases, further investigations using molecular methods were conducted urgently to allow tissue to be used. In each case, the S/CO ratio in the screening assay (not necessarily the Abbott Architect rHTLV-I/II) was low, and in each case, the final diagnosis was HTLV-1/-2-uninfected.

The goal of this study was to determine the risk of HTLV infection in individuals whose serum was tested on the Abbott Architect rHTLV-I/II platform and found to have low titre reactivity. The primary finding of this study of more than 10 000 individual blood results is that on no occasion has an individual whose sample gave an S/CO ratio of less than 4.0 in the Abbott Architect rHTLV-I/II been shown, on further investigation, to be HTLV-1- or HTLV-2-infected. Thus, where an HTLV assay is urgently conducted to protect a tissue transplantation recipient from HTLV infection and the S/CO ratio is less than 4.0, it is reasonable to advise the transplantation team and the recipient that the likelihood that the donor is truly infected with HTLV-1 is low and that although HTLV-1 infection cannot be fully eliminated until further investigations have been completed, using the tissue is a legitimate option within the context of an appropriate risk–benefit assessment.

The focus of this paper has been on transplantation due to the short window for serological evaluation. The interpretations of the data are equally applicable to testing for other indications

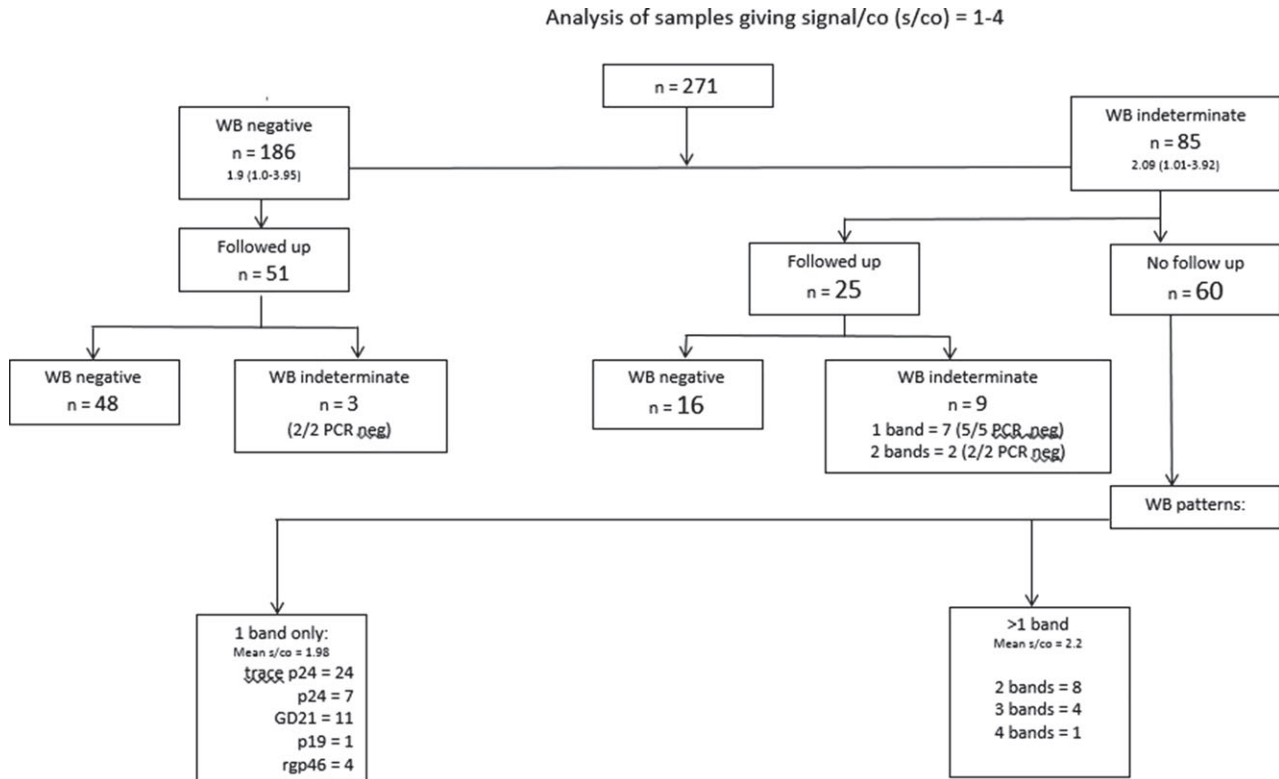


Fig. 3. Flow chart summarising the outcomes of samples with a signal/cut-off ratio of 1 < 4.

where the need for further evaluation of low-level reactivity should be considered on its merits. The cost of evaluating 'false positives' is cited as a contraindication for screening. If the number of 'false positives' can be reduced by the suggested adjustment, this would have implications for other screening programmes, including blood and antenatal.

There are a number of limitations to the study. The findings are only applicable to the Abbott Architect rHTLV I/II platform, although this is one of the most widely used platforms in the UK. Only 271 samples with an initial Abbott Architect result in the S/CO ratio range 1–4 were identified resulting in a 2% margin of error in the 95% confidence interval for the PPV. Thirdly, a final diagnosis of 'HTLV indeterminate' remained in 113 cases due to lack of follow-up samples to allow molecular detection assays. In particular, in the key S/CO ratio range 1–4, this was the case in 69 of the 271 samples, and therefore, it remains possible that one or more of these might represent a true positive. However, given that none of the 36 samples that were initially indeterminate in

the range 1–20 and had follow-up sample for repeat Western blot and/or HTLV DNA PCR were found to be true positives, it is considered unlikely that the number of true positives would exceed the 95% CI of the PPV.

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CONFLICT OF INTEREST

The authors have no competing conflicts of interest.

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