Comparison of two new HTLV-I/II screening methods, Abbott Alinity i rHTLV-I/II and Diasorin LIAISON® XL murex recHTLV-I/II, to Abbott Architect rHTLVI/II assay

Vincent Guiraud, Florian Crémoux, Isabelle Leroy, Julien Cohier, Pierre Hernandez, Safietou Mansaly, Agnès Gautheret-Dejean

Comparison of two new HTLV-I/II screening methods, Abbott Alinity I rHTLV-I/II and Diasorin LIAISON® XL murex recHTLV-I/II, to Abbott architect rHTLVI/II assay

Vincent Guiraud[a,&], Florian Crémoux[a,&], Isabelle Leroy[a], Julien Cohier[a], Pierre Hernandez[a], Safietou Mansaly[a], Agnès Gautheret-Dejean[a,b,*]

[a] *AP-HP, Hôpitaux Universitaires La Pitié Salpêtrière-Charles Foix, Service de Virologie, F-75013 Paris, France*

[b] *Université Paris cité, INSERM UMR-S 1139 Physiopathologie et pharmacotoxicologie placentaire humaine : microbiote pré & post-natal, F-75006 Paris, France*

[&] *These authors also contributed equally to this work.*

* Corresponding author: Hôpital Universitaire La Pitié Salpêtrière, Service de Virologie, 83 Bd de l'Hôpital, 75013 Paris, France. Tel: +33 1 42177401 / Fax: +33 1 42177411. E-Mail: agnes.gautheret@aphp.fr

ABSTRACT

Background: Diagnosis of Human T-cell Lymphotropic Virus (HTLV) types I and II infection requires sequencial testing with firstly a screening using an Enzyme immunoassay followed by a confirmatory test.

Objectives: To compare the performances of the Alinity i rHTLV-I/II (Abbott®) and LIAISON® XL murex recHTLV-I/II serological screening tests to the ARCHITECT rHTLVI/II test followed if positive by HTLV BLOT 2.4, MP Diagnostics as the reference.

Study design: 119 serum samples from 92 known HTLV-I infected patients and 184 from uninfected patients with HTLV were analyzed in parallel with, Alinity i rHTLV-I/II, LIAISON® XL murex recHTLV-I/II and ARCHITECT rHTLVI/II.

Results: Alinity i rHTLV-I/II and Liaison XL murex recHTLV-I/II exhibited a total agreement with ARCHITECT rHTLVI/II for both positive and negative samples. Both tests are suitable alternatives for HTLV screening.

Keywords:

Human T-cell lymphotropic virus, serological assay, method comparison, ARCHITECT rHTLVI/II, Alinity i rHTLV-I/II, LIAISON® XL murex recHTLV-I/II

38 Abbreviations:

39 HTLV: Human T-cell Lymphotropic Virus, 95% CI: 95% Confidence interval, ATLL: adult
40 T-cell leukemia/lymphoma, HAM: HTLV-associated myelopathy, CMIA: chemiluminescent
41 microparticle immunoassay, CLIA: chemiluminescent immunoassay, RLU: Relative Light
42 Unit, S/CO: signal/cutoff, CV: coefficient of variation, SD: standard deviations.

43

1. Background

Human T lymphotropic virus type I (HTLV-I) and Human T lymphotropic virus type II (HTLV-II) were the first retroviruses discovered, respectively in 1980 [1] and 1982 [2]. They are responsible for adult T-cell leukemia/lymphoma (ATLL) [3], HTLV-associated myelopathy (HAM) [4] and inflammatory diseases such as uveitis, myositis and dermatitis [4]. HTLV in adult population is unevenly distributed worldwide: highly endemic in Japan, Caraibeans and several African areas while virtually absent in other regions [5,6]. However, in both low and high endemic countries prevalence may exhibit discrepancies. For instance areas in Texas or Nevada has seroprevalence more than ten times higher than other parts of the United States [7]. HTLV transmission occurs in utero, peripartum mostly through breastfeeding [8], during sexual relationship [9], intravenous drug use [10], and solid organ or hematopoietic transplantation [11]. Finally, although blood transfusion has been an historical route of HTLV transmission, leukofiltration has significantly reduced this risk [12].

To limit blood and graft-born transmission, 35 countries to date test each donor for HTLV serology, and 15 more implement selective testing according to specific risk factors [13]. Since serological screening is both the most sensitive [14] and cost-effective way to screen [15] but lack specificity, guidelines recommend a dual-testing algorithm with firstly a third-generation screening using chemiluminescent immunoassay or enzyme-linked immunosorbent assay. In case of reactive or indeterminate result, a specific confirmatory assay with an Immunoblot, Western blot or line immunoassay [16,17], is performed.

2. Objectives

The objective of this study was to compare two screening immunoassays, Alinity i rHTLV-I/II (Abbott®, Rungis, France), LIAISON® XL murex recHTLV-I/II (DiaSorin, Antony, France), with ARCHITECT rHTLV-I/II (Abbott, Rungis, France) followed for positive

samples by HTLV BLOT 2.4 (MP Diagnostics™, Illkirch-Graffenstaden, France) as the reference.

3. Study design

3.1 Samples

The study included a retrospective part of clinical and analytical sensitivity, and an exploratory prospective part of specificity. All serum samples had been tested following the routine algorithm of the Service of Virology at the Pitié Salpêtrière Hospital (Paris, France). Architect rHTLV-I/II was used as the screening method with HTLV BLOT 2.4 as the confirmatory assay for reactive results. Only samples confirmed HTLV positive were included in the retrospective study. For clinical sensitivity, 119 serum samples from 92 HTLV-I subjects were included. Serum samples were stored at -20°C before use. Analytical sensitivity was done using dilutions of positive serum samples from two HTLV-I infected patients. For the exploratory specificity prospective study, 184 fresh non-reactive serum samples using Architect rHTLV-I/II were included from January 4th 2022 to March 1st 2023. They were stored at +4°C before use. Reproducibility and repeatability were assessed with positive and negative controls of the kits.

3.2 Methods and analysis

For clinical sensitivity, all samples were tested the same day according to the manufacturer's instructions with the three methods: Architect rHTLV-I/II, Alinity i rHTLV-I/II and LIAISON® XL murex recHTLV-I/II. Architect rHTLV-I/II and Alinity i rHTLV-I/II are based on chemiluminescent microparticle immunoassay (CMIA) and LIAISON® XL murex recHTLV-I/II is based on chemiluminescent immunoassay (CLIA). All methods use HTLV-I/II gp46 synthetic peptides and HTLV-I p21 recombinant protein. In addition, LIAISON® XL murex recHTLV-I/II contains HTLV-II p21 recombinant protein. Results for all three

methods were expressed as the ratio of the Relative Light Unit (RLU) of the sample (signal) to the RLU of the cutoff (S/CO). A reactive result was defined as a ratio ≥ 1 and a non-reactive result was defined as a ratio <1. Of note, none of the three assays allocated undetermined results.

To evaluate repeatability, positive and negative controls were tested 23 times in the same run following previous guidelines [18]. Reproducibility was assessed by testing positive and negative controls of the kit once a run for 30 consecutive days. Repeatability and reproducibility were evaluated by analyzing coefficients of variation (CV), defined as the ratio of standard deviation of the S/CO value to the mean S/CO value.

Dilution for analytical sensitivity analyses were: 1:10, 1:100, 1:200, 1:500, 1:1000, 1:10000. The three methods were performed in parallel.

For the prospective exploratory specificity study, 184 non-reactive samples using Architect rHTLV-I/II were tested with LIAISON® XL murex recHTLV-I/II and Alinity i rHTLV-I/II in parallel the same day.

3.3 Statistical analysis

Statistical analyses were conducted using R version 4.2.1 [19]. S/CO values were compared using Spearman correlation coefficient (r) and Wilcoxon's test for paired data. All reported P values are two-sided, with $p < 0.05$ considered statistically significant. Delta (δ) value was used to estimate the methods' abilities to separate reactive and non-reactive populations from the cut off [20–22]. Delta value was defined for both reactive and non-reactive population as the distance between the population mean and the CO, expressed in standard deviation (SD) units of the log transformed population distribution according to the following formula:

$$Delta\ (\delta) = \frac{Population\ mean\ (\log(S/CO))}{SD\ (\log(S/CO))}$$

4. Results

With ARCHITECT rHTLVI/II followed for reactive sera by HTLV BLOT 2.4, MP Diagnostics as the reference assay, Alinity i rHTLV-I/II and LIAISON® XL murex recHTLV-I/II identified correctly all 119 positive samples leading to a sensitivity of 100% with a 95% CI [97%-100%]. All S/CO ranges were similar for the three methods, but Alinity i rHTLV-I/II had a mean S/CO value lower in comparison with Architect (p = 7.5E-16) and Liaison (p = 1.2E-11) (Table 1). Mean S/CO value for reactive samples was higher for Liaison murex recHTLV-I/II than Architect rHTLV-I/II (p = 0.045). For 92 serum samples, S/CO values were lower using Alinity i rHTLV-I/II in comparison with the other two methods. Reactive S/CO values were well correlated between ARCHITECT rHTLV-I/II and Alinity i rHTLV-I/II (R^2 = 0.53, p < 2.2E-16), but less correlated between ARCHITECT rHTLV-I/II and Liaison XL (R^2 = 0.26, p = 6.5E-9) or Alinity i rHTLV-I/II and LIAISON® XL murex recHTLV-I/II (R^2 = 0.22, p = 9.9E-8) (Fig. 1). A phenomenon of saturation was observed for LIAISON® XL murex recHTLV-I/II for S/CO values above 130 when Architect rHTLV-I/II positivity was over 75 IU (Fig. 1).

Results of sensitivity for endpoint dilutions were similar for the three methods, with a negativation around 1:10000 for patient 1, and around 1:200 for patient 2 (reported in Supplemental Table 1).

Results of reproducibility were similar for all three methods, with CV for positive control ranging from 4.7% (95% CI [3.7%-6.4%]) for Alinity i rHTLV-I/II to 9.6% (95% CI [7.7%-13.1%]) for LIAISON® XL murex recHTLV-I/II Assays, and for negative control ranging from 5.7% (95% CI [4.7-7.7]) to 20.1% (95% CI [16.0-27.2]) (reported in Supplemental Table 2). Results of repeatability were also similar for all three methods, with CV for positive

control ranging from 1.3% (95% CI [1.0-1.7]) for Alinity i rHTLV-I/II to 3.7% (95% CI [3.0-5.1]) for ARCHITECT rHTLV-I/II. For negative controls, CV ranged from 5.7% (95% CI [4.5-7.7]) for LIAISON® XL murex recHTLV-I/II to 10.7% (95% CI [8.3-15.1]) for Alinity i rHTLV-I/II. None of the positive and negative controls tested was misclassified.

Of note, for the exploratory study of specificity, with ARCHITECT rHTLV-I/II as the reference assay, Alinity i rHTLV-I/II and LIAISON® XL murex recHTLV-I/II identified correctly the 184 negative serum samples.

The ability for each test to allow a high discrimination between reactive and non-reactive populations was assessed by the δ coefficient with the higher absolute value the better discrimination. Interestingly, compared with ARCHITECT rHTLV-I/II (-4.8 for non-reactive and 8.8 for reactive), Alinity i rHTLV-I/II seemed to be less discriminant assay (-9.1 for non-reactive and 7.9 for reactive) for reactive samples, while LIAISON® XL murex recHTLV-I/II might be the most discriminant (-9.5 for non-reactive and +13.9 for reactive) (Fig. 2).

5. Discussion

All 119 samples from HTLV-I-infected patients were tested positive with the two studied methods, although S/CO values were moderately correlated. A phenomenon of saturation of the signal was observed with LIAISON® XL murex recHTLV-I/II above 130 S/CO, and positive ratios were lower using Alinity i rHTLV-I/II. Of note, all 184 uninfected patients were non-reactive with the two methods. Positive and negative results were highly discriminated, especially for LIAISON® XL murex recHTLV-I/II with a δ coefficient at -9.5 for non-reactive and +13.9 for reactive samples.

This study is in agreement with the study of Malm et al. where 38 positive samples were tested with ARCHITECT rHTLV-I/II and Murex recHTLV-I/II EIA method in a microplate formate used as the reference method [23]. Similar results were obtained by Qiu et al. on 498

samples from patients infected by HTLV-I/II that were tested in parallel with ARCHITECT rHTLV-I/II and murex recHTLV-I/II in microplate [21]. Our results differed from those obtained by Gantner et al. who reported, on a panel of 66 samples, a sensitivity of LIAISON® XL murex recHTLV-I/II at 78% when comparing with the results obtained with ARCHITECT rHTLV-I/II [24]. However this study included positive samples based on low Architect S/CO values as a reference without confirmatory assay and so may have introduced a classification bias due to the high proportion of false-positive for low Architect S/CO positive values [25]. Surprinsingly, to date no other study have compared sensitivities of the Alinity assay to another platform.

On a limited number of samples, our study found a specificity of 100% for the three methods. These values are within the range of previous published studies, which found a specificity at 99.98% with 95%CI [99.92%-100%] for ARCHITECT rHTLV-I/II [21], at 99.4% with 95%CI [98.3%-99.8%] for ARCHITECT rHTLV-I/II [23], at 99.5% with 95%CI [98.0-99.9] for LIAISON® XL murex recHTLV-I/II [25] and 99.92% for the Alinity assay [26].

The main limitation of our study was the use of the Architect assay as the screening reference. As a consequence, when testing pure serum samples, we were not able to determine if any of the other tests were more sensitive than the Architect. This point was slightly evaluated with end-point dilutions although results were roughly identical for the three techniques. Moreover, as we had a limited number of negative samples tested, we may have lacked power to rank the assays according to their specificities and had to use the delta coefficient as a surrogate marker. Finally, as we defined positive sample as both ARCHITECT rHTLV-I/II and HTLV BLOT 2.4 positivity we may have missed patients with low reactivity, and as we managed to include only HTLV-I positive samples our results should be taken with caution depending on local epidemiology.

6. Conclusion

On a serum panel of 119 infected and 184 uninfected patients with HTLV-I/II, Alinity i rHTLV-I/II and LIAISON® XL murex recHTLV-I/II exhibited a total agreement compared with Architect rHTLV-I/II as the referent assay. These two tests are therefore suitable for the screening of HTLV-I/II infection in donors, subject at risk and patients with ATLL or HAM.

Author contributions

Vincent Guiraud data analyzes, writing- original draft preparation, submission of the final manuscript. Florian Crémoux supervision, acquisition and analyzes of data, validation. Isabelle Leroy visualization, samples testing. Julien Cohier samples testing. Pierre Hernandez samples testing. Safietou Mansaly samples testing and Agnès Gautheret-Dejean conceptualization, methodology, writing- reviewing and editing.

Fundings

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interests

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments

The authors thank ADEBIOPHARM ER28 association for the financial participation to this study. The authors thank Abbott and DiaSorin laboratories for providing free kits for this study. Otherwise, they had no part on study design, data collection, data analyses and interpretation, manuscript writing nor submission to publication. The authors thank Mostafa Habib for his assistance.

Ethics

This study complies with Good Clinical Practices and ethical principles of the Helsinki declaration. All data were anonymized before analysis. Patients were systematically notified of any supplementary biological analyses on frozen samples, initially collected as part of routine clinical practice.

References

[1] B.J. Poiesz, F.W. Ruscetti, A.F. Gazdar, P.A. Bunn, J.D. Minna, R.C. Gallo, Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma, Proc. Natl. Acad. Sci. 77 (1980) 7415–7419. https://doi.org/10.1073/pnas.77.12.7415.

[2] V.S. Kalyanaraman, M.G. Sarngadharan, M. Robert-Guroff, I. Miyoshi, D. Blayney, D. Golde, R.C. Gallo, A New Subtype of Human T-Cell Leukemia Virus (HTLV-II) Associated with a T-Cell Variant of Hairy Cell Leukemia, Science. 218 (1982) 571–573. https://doi.org/10.1126/science.6981847.

[3] A. Utsunomiya, I. Choi, D. Chihara, M. Seto, Recent advances in the treatment of adult T-cell leukemia-lymphomas, Cancer Sci. 106 (2015) 344–351. https://doi.org/10.1111/cas.12617.

[4] F. Martin, G.P. Taylor, S. Jacobson, Inflammatory manifestations of HTLV-1 and their therapeutic options, Expert Rev. Clin. Immunol. 10 (2014) 1531–1546. https://doi.org/10.1586/1744666X.2014.966690.

[5] A. Gessain, O. Cassar, Epidemiological Aspects and World Distribution of HTLV-1 Infection, Front. Microbiol. 3 (2012). https://doi.org/10.3389/fmicb.2012.00388.

[6] European Centre for Disease Prevention and Control., Geographical distribution of areas with a high prevalence of HTLV-1 infection., Publications Office, LU, 2015. https://data.europa.eu/doi/10.2900/047633 (accessed February 7, 2023).

[7] Y.B. Chang, Z. Kaidarova, D. Hindes, M. Bravo, N. Kiely, H. Kamel, D. Dubay, B. Hoose, E.L. Murphy, Seroprevalence and Demographic Determinants of Human T-Lymphotropic Virus Type 1 and 2 Infections Among First-Time Blood Donors--United States, 2000-2009, J. Infect. Dis. 209 (2014) 523–531. https://doi.org/10.1093/infdis/jit497.

[8] S. Hino, S. Katamine, K. Kawase, T. Miyamoto, H. Doi, Y. Tsuji, T. Yamabe, Intervention of maternal transmission of HTLV-1 in Nagasaki, Japan, Leukemia. 8 Suppl 1 (1994) S68-70.

[9] D.F. Roucoux, B. Wang, D. Smith, C.C. Nass, J. Smith, S.T. Hutching, B. Newman, T. Lee, D.M. Chafets, E.L. Murphy, HTLV Outcomes Study Investigators, A Prospective Study of Sexual Transmission of Human T Lymphotropic Virus (HTLV)–I and HTLV-II, J. Infect. Dis. 191 (2005) 1490–1497. https://doi.org/10.1086/429410.

[10] M. Salemi, A.-M. Vandamme, C. Gradozzi, K. Van Laethem, E. Cattaneo, G. Taylor, C. Casoli, P. Goubau, J. Desmyter, U. Bertazzoni, Evolutionary Rate and Genetic Heterogeneity of Human T-Cell Lymphotropic Virus Type II (HTLV-II) Using Isolates from European Injecting Drug Users, J. Mol. Evol. 46 (1998) 602–611. https://doi.org/10.1007/PL00006340.

[11] on behalf of the Spanish HTLV Network, C. de Mendoza, L. Roc, R. Benito, G. Reina, J.M. Ramos, C. Gómez, A. Aguilera, M. Rodríguez-Iglesias, J. García-Costa, M. Fernández-Alonso, V. Soriano,

HTLV-1 infection in solid organ transplant donors and recipients in Spain, BMC Infect. Dis. 19 (2019) 706. https://doi.org/10.1186/s12879-019-4346-z.

[12] R. Sobata, C. Matsumoto, S. Uchida, Y. Suzuki, M. Satake, K. Tadokoro, Estimation of the infectious viral load required for transfusion-transmitted human T-lymphotropic virus type 1 infection (TT-HTLV-1) and of the effectiveness of leukocyte reduction in preventing TT-HTLV-1, Vox Sang. 109 (2015) 122–128. https://doi.org/10.1111/vox.12263.

[13] World Health Organization, Global status report on blood safety and availability 2021, World Health Organization, Geneva, 2022. https://apps.who.int/iris/handle/10665/356165 (accessed October 6, 2022).

[14] K.R. Campos, M.G. Gonçalves, N.A. Costa, A. Caterino-de-Araujo, Comparative performances of serologic and molecular assays for detecting human T lymphotropic virus type 1 and type 2 (HTLV-1 and HTLV-2) in patients infected with human immunodeficiency virus type 1 (HIV-1), Braz. J. Infect. Dis. 21 (2017) 297–305. https://doi.org/10.1016/j.bjid.2017.02.005.

[15] E.L. Murphy, Infection with human T-lymphotropic virus types-1 and -2 (HTLV-1 and -2): Implications for blood transfusion safety, Transfus. Clin. Biol. 23 (2016) 13–19. https://doi.org/10.1016/j.tracli.2015.12.001.

[16] Use of Serological Tests to Reduce the Risk of Transfusion-Transmitted Human T-Lymphotropic Virus Types I and II (HTLV-I/II) - Guidance for industry, (2020). https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-serological-tests-reduce-risk-transfusion-transmitted-human-t-lymphotropic-virus-types-i-and-ii.

[17] E.C. Sabino, M. Zrein, C.P. Taborda, M.M. Otani, G. Ribeiro-Dos-Santos, A. Sáez-Alquézar, Evaluation of the INNO-LIA HTLV I/II Assay for Confirmation of Human T-Cell Leukemia Virus-Reactive Sera in Blood Bank Donations, J. Clin. Microbiol. 37 (1999) 1324–1328. https://doi.org/10.1128/JCM.37.5.1324-1328.1999.

[18] R.H. Jacobson, Validation of serological assays for diagnosis of infectious diseases: -EN- -FR- Validation des épreuves sérologiques pour le diagnostic des maladies infectieuses -ES-

Validación de pruebas serológicas para el diagnóstico de enfermedades infecciosas, Rev. Sci. Tech. OIE. 17 (1998) 469–526. https://doi.org/10.20506/rst.17.2.1119.

[19] R Core Team, R: A Language and Environment for Statistical Computing, (n.d.).

[20] N. Crofts, W. Maskill, I.D. Gust, Evaluation of enzyme-linked immunosorbent assays: a method of data analysis, J. Virol. Methods. 22 (1988) 51–59. https://doi.org/10.1016/0166-0934(88)90087-0.

[21] W.J. Maskill, N. Crofts, E. Waldman, D.S. Healey, T.S. Howard, C. Silvester, I.D. Gust, An evaluation of competitive and second generation ELISA screening tests for antibody to HIV, J. Virol. Methods. 22 (1988) 61–73. https://doi.org/10.1016/0166-0934(88)90088-2.

[22] X. Qiu, S. Hodges, T. Lukaszewska, S. Hino, H. Arai, J. Yamaguchi, P. Swanson, G. Schochetman, S.G. Devare, Evaluation of a new, fully automated immunoassay for detection of HTLV-I and HTLV-II antibodies, J. Med. Virol. 80 (2008) 484–493. https://doi.org/10.1002/jmv.21083.

[23] K. Malm, T. Kjerstadius, S. Andersson, Evaluation of a new screening assay for HTLV-1 and -2 antibodies for large-scale use, J. Med. Virol. 82 (2010) 1606–1611. https://doi.org/10.1002/jmv.21867.

[24] P. Gantner, A. Velay, N. Guigue, H. Barth, M.-J. Wendling, C. Delaugerre, S. Fafi-Kremer, Performance of the Liaison® XL Murex recHTLV-I/II Immunoassay in the Detection of HTLV-1/2 Antibodies in Serum, Clin. Lab. 63 (2017). https://doi.org/10.7754/Clin.Lab.2017.161026.

[25] J.H.C. Tosswill, G.P. Taylor, Interpretation of low reactivity in the Abbott Architect rHTLV I/II assay: Interpretation of low level HTLV reactivity, Transfus. Med. 28 (2018) 326–330. https://doi.org/10.1111/tme.12482.

[26] Vokel A, Chen G, Bui T, Specificity of Automated Blood Screening Assays developed for the new Abbott ALINITY S System, (n.d.).

Table 1

Distribution of S/CO values[1] for positive and negative samples with Architect, Alinity and Liaison assays.

Type of samples		**Architect**	**Alinity**	**Liaison**
Positive samples	S/CO≥1	119	119	119
(n=119)	Minimum	17.4	11.2	26
	Mean	118.4	100.3	130
	Standard deviation	49.1	44.0	31.3
	Maximum	241.4	225.7	190
Negative samples	S/CO<1	184	184	184
(n=184)	Minimum	0.07	0.05	0.21
	Mean	0.19	0.10	0.28
	Standard deviation	0.066	0.030	0.045
	Maximum	0.47	0.28	0.67

[1] S/CO, signal/cut off.

317 **Supplemental Table 1**

318 Values of S/CO[1] according to sera dilutions for two HTLV-I-infected patients. Last detectable
319 dilutions (end-point dilutions) are in bold underlined.

Serum dilution	Results for patient 1 with			Results for patient 2 with		
	Architect	**Alinity**	**Liaison**	**Architect**	**Alinity**	**Liaison**
1:1	82.32	76.74	140	62.84	60.37	71
1:10	40.7	39.7	73	8.45	**8.35**	**7.40**
1:100	7.51	7.99	18	**1.01**	0.95	0.99
1:200	3.89	4.16	10	0.56	0.52	0.62
1:500	**1.76**	**1.76**	4.5	0.32	0.25	0.36
1:1000	0.96	0.98	**2.4**	0.22	0.18	0.30
1:10000	0.2	0.16	0.45	0.15	0.08	0.26

320 [1] S/CO, signal/cut off. S/CO ≥ 1 are positive. Values in bold underlined
321 correspond to the last positive value for each method. Due to low sample
322 volume we were unable to do replicates.

323

324

Supplemental table 2

Reproducibility and repeatability for positive controls with Architect, Alinity and Liaison assays.

	Results for positive and negative controls with		
	Architect	**Alinity**	**Liaison**
Positive controls			
Reproducibility (min-max)	6.9 (5.6-9.4)[1]	4.7 (3.7-6.4)[1]	9.6 (77-13.1)[1]
Repeatability (min-max)	3.7 (3-5.1)	1.3 (1-1.7)	2.3 (1.9-3.2)
Negative controls			
Reproducibility (min-max)	6.9 (5.6-9.4)	6.9 (5.6-9.4)	6.9 (5.6-9.4)
Repeatability (min-max)	6.9 (5.6-9.4)	6.9 (5.6-9.4)	6.9 (5.6-9.4)

[1] Results are expressed as Coefficient of variation (%) (95% CI). 95% CI, 95% confidence interval.

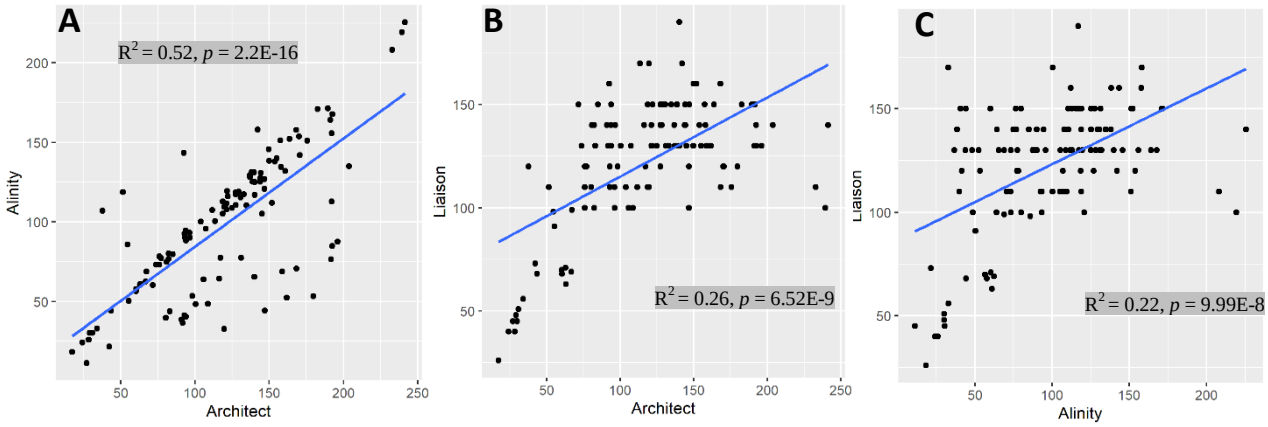

Figure 1: S/CO and correlation for reactive samples for Architect and Alinity (A), Architect and Liaison (B) and Alinity and Liaison (C).