Supplementary information
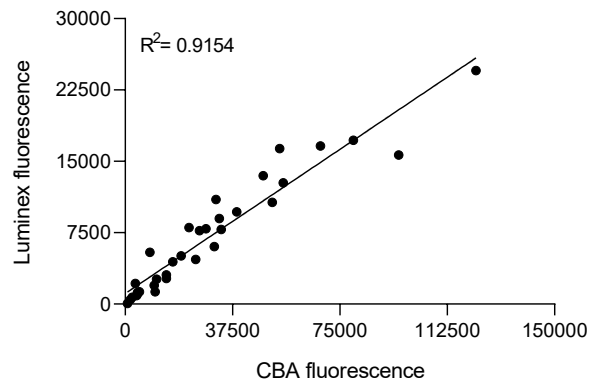
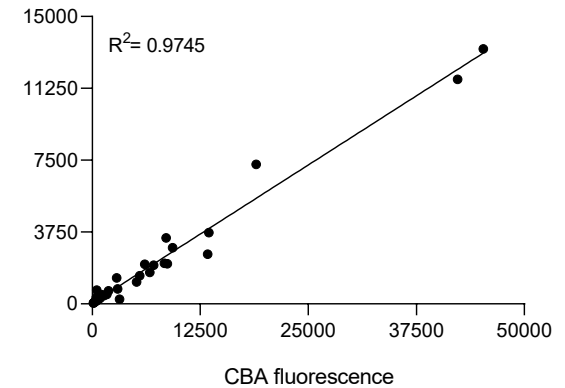

A ASO
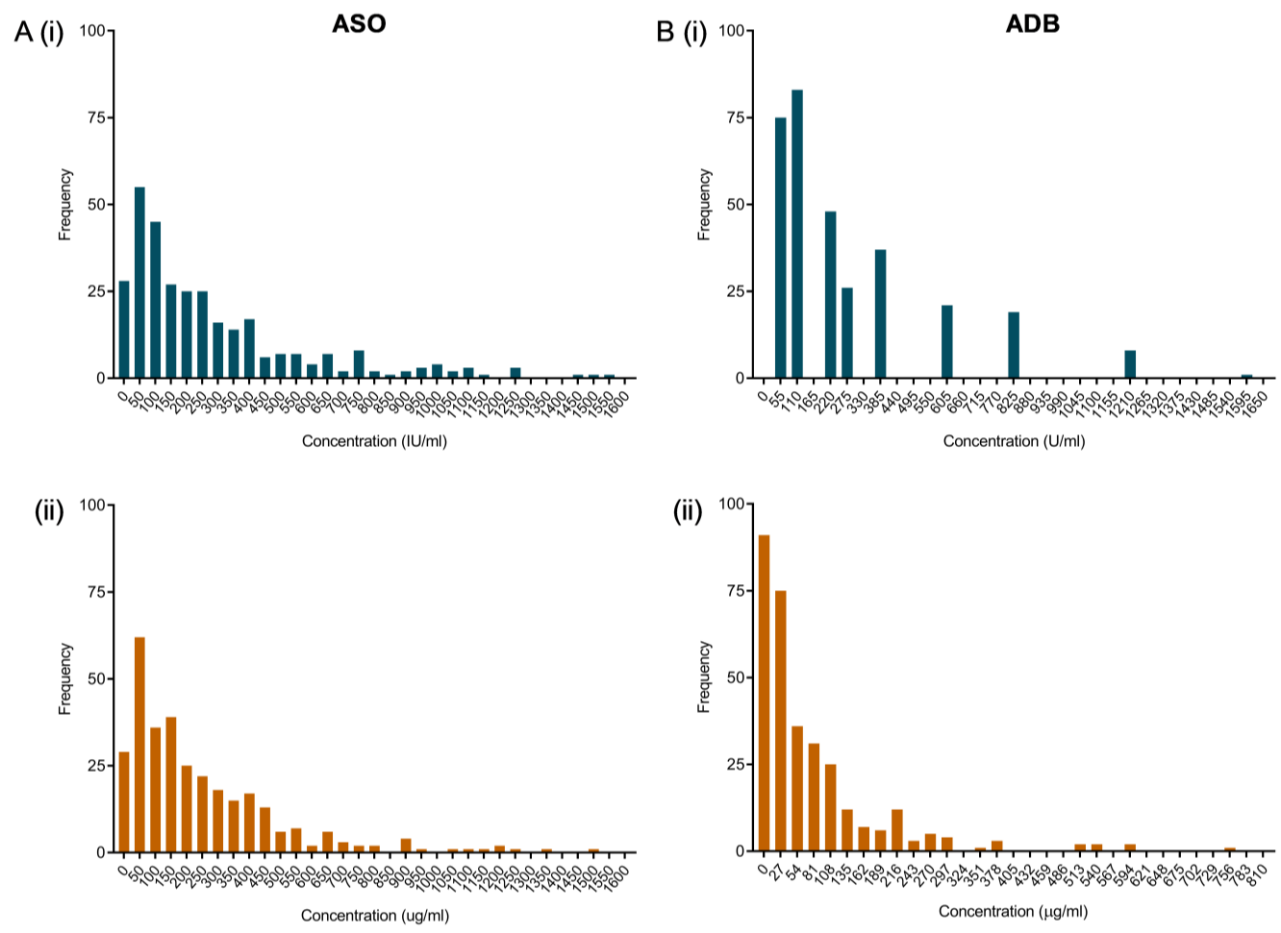
B ADB

C Anti-SpnA

$R^2= 0.9154$

$R^2= 0.9341$

$R^2= 0.9745$

Supplementary Figure 1: Scatter plots showing the correlation of (A) ASO, (B) ADB and (C) Anti-SpnA signal, in fluorescence, between the Luminex assay and the cytometric bead array (CBA) (n=33). Strength of correlation is shown as R^2, obtained by linear regression.

Supplementary Figure 2: (A) Distribution of ASO antibodies (n=317), determined either by the (i) commercial turbidimetric assay or the (ii) triplex assay. (B) Distribution of ADB antibodies (n=318), determined either by the (i) commercial enzyme inhibition assay or the (ii) triplex assay.

Supplementary Table 1: ULN values determined from various healthy cohorts, including the current guidelines used in clinical practice in Australia and New Zealand. The original units of each ULN are highlighted in blue and reflect the method used to obtain them. Values were interpolated into either µg/ml, IU/ml or U/mL using the linear regression equations from Figure 1 where $r^2=0.803$ for ASO and $r^2=0.782$ for ADB, to allow comparison between methods.

Year	ULN	Method	ASO (IU/ml)	ADB (U/ml)	ASO (µg/ml)	ADB (µg/ml)	Anti-SpnA (µg/ml)
2019[i]	NZ RF RISK experimental, all age	Triplex assay	412	367	354	117	64
2019[ii]	NZ RF RISK, all age	Commercial assays	422	400	353	127	-
1982[iii]	NZ, all age	Commercial assays	480	680	398	225	-
2012[iv]	AUS 1-4	Commercial assays	170	366	158	115	-
	AUS 5-14		276	499	240	161	-
	AUS 15-24		238	473	211	152	-
	AUS 25-34		177	390	163	123	-
	AUS >35		127	265	125	79	-
2006[v]	AUS 4-5	Commercial assays	120	100	119	21	-
	AUS 6-9		480	400	398	127	-
	AUS 10-14		320	380	274	120	-

(i)   ULN calculated using titres measured by the triplex assay, from healthy New Zealand children in the RF RISK study (n=75)
(ii)   ULN calculated from titres measured using commercial assays, from healthy New Zealand children in the RF RISK study (n=75)
(iii)   Current recommended clinical cut-off used in New Zealand for streptococcal serology, calculated from New Zealand children <15 years old, who were at-risk of ARF but hospitalised for other reasons[1]
(iv)   Current age-specific recommended clinical cut-offs used in Australia for streptococcal serology, calculated from healthy Fijian participants (n=424)[2]
(v)   Age-specific recommended clinical cut-offs used in Australia for streptococcal serology in 2006, calculated from healthy children in Melbourne, Australia (n=66)[3]

Supplementary Table 2: Comparison of diagnostic sensitivity of ASO and ADB when different ULN are applied, with sensitivity defined as the number of ARF patients with antibody titres above the ULN. ARF patients were stratified into those with sera obtained <28 days after hospital admission and 28+ after hospital admission. Current clinical ULN values from New Zealand and Australia were first interpolated into µg/ml using the linear regression equations from Figure 1 where $r^2=0.803$ for ASO and $r^2=0.782$ for ADB.

	ASO			ADB		
	Experimental ULN	NZ all-age ULN[1]	AUS age-specific ULN[2]	NZ all-age experimental	NZ all-age ULN	AUS age-specific ULN
ARF < 28 days, n= 72, n (%)	63 (88)	61 (85)	70 (97)	63 (88)	40 (56)	54 (75)
ARF 28 + days, n=25, n (%)	23 (92)	22 (88)	24 (96)	24 (96)	12 (48)	20 (80)

References

1. Heart Foundation of New Zealand, Cardiac Society of Australia and New Zealand. Evidence-based, best practice New Zealand Guidelines for Rheumatic Fever: 1. Diagnosis, management and secondary prevention of Acute Rheumatic Fever and Rheumatic Heart Disease: 2014 Update. Heart Foundation of New Zealand, 2014.

2. Steer AC, Vidmar S, Ritika R, Kado J, Batzloff M, Jenney AWJ, et al. Normal Ranges of Streptococcal Antibody Titers Are Similar Whether Streptococci Are Endemic to the Setting or Not. Clin Vaccine Immunol. 2009;16:172–5.

3. Danchin MH, Carlin JB, Devenish W, Nolan TM, Carapetis JR. New normal ranges of antistreptolysin O and antideoxyribonuclease B titres for Australian children. Journal of Paediatrics and Child Health. 2005;41:583–6.