

1 **Africa-wide evaluation of host biomarkers in QuantiFERON supernatants for the**
2 **diagnosis of pulmonary tuberculosis:**

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4 **Supplementary Results**

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62 **Supplementary Table 1: Median levels of individual analytes (pg/ml) in HIV uninfected**
 63 **study participants (n=135 TB Vs. n=223 ORD) and accuracies in the diagnosis of TB**
 64 **disease as determined by area under the ROC curve.** Only analytes with p-values <0.05 in
 65 the Mann Whitney U test are shown. IQR= Inter-quartile range, AUC = area under the ROC
 66 curve, _N= Unstimulated levels, _{Ag-N} = antigen specific levels, *The negative median values
 67 indicate that the value obtained in the antigen stimulated tube was lower than the value obtained
 68 in the nil tube and this phenomenon has been observed in previous studies¹.

Analyte	TB (IQR)	ORD (IQR)	P-value	AUC (95% CI)
IL-1ra_N	386.7(200.7-646.5)	295.5(159.2-525.4)	0.04	0.59(0.51-0.67)
VEGF_N	102.8(0.0-226.0)	55.4(0.0-119.0)	0.002	0.61(0.55-0.68)
IFN-γ_N	17.79(9.0-34.9)	6.1(1.9-15.30)	<0.0001	0.73(0.68-0.78)
IFN-α2_N	36.2(17.0-53.4)	20.0(7.7-39.3)	0.001	0.64(0.56-0.72)
sCD40L_N	2625(1387-4815)	1434(835.8-2701)	<0.0001	0.67(0.61-0.73)
MIP-1β_N	1000(418.3-2132)	1324(684.9-2699)	0.003	0.59(0.53-0.66)
TGF-α_N	20.0(12.9-31.0)	10.2(4.2-19.1)	<0.0001	0.69(0.64-0.75)
MMP-2_N	119653(75661-152183)	123161(102812-164515)	0.042	0.56(0.50-0.63)
MMP-9_N	910512(516362-1.344x10 ⁶)	540324 (301475-899608)	<0.0001	0.69(0.63-0.75)
IL-1ra_{Ag-N}	241.2(69.7-1002.0)	102.2(-10.7-400.2)	0.005	0.62(0.54-0.71)
*VEGF_{Ag-N}	-20.9 (-102.5-0.0)	-0.0(-36.7-0.0)	0.006	0.59(0.52-0.65)
IFN-γ_{Ag-N}	151.4(29.8-438.7)	31.1(2.6-217.8)	<0.0001	0.64(0.58-0.70)
*TGF-α_{Ag-N}	1.3(-1.9-7.9)	0.0(-1.6-3.1)	0.023	0.57(0.50-0.64)
*MMP-9_{Ag-N}	75895(-36060-289983)	19103(-81433-121961)	0.005	0.60(0.53-0.66)

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75 **Supplementary Table 2: Median levels of individual analytes (pg/ml) obtained in the**
 76 **definite TB cases (n=160) and accuracies in the discriminating between definite TB and**
 77 **other respiratory diseases (n=274) regardless of HIV infection status.** Only analytes with
 78 p-values <0.05 in the Mann Whitney U test are shown. IQR= Inter-quartile range, AUC = area
 79 under the ROC curve, _N= Unstimulated levels, _{Ag-N} = antigen specific levels. *The negative
 80 median values indicate that the value obtained in the antigen stimulated tube was lower than
 81 the value obtained in the nil tube and this phenomenon has been observed in previous studies
 82 ¹, #not significant; marker shown because it was one of the promising markers identified in
 83 our previous small study¹.

ANALYTE	TB (IQR)	ORD (IQR)	P-VALUE	AUC (95% CI)
VEGF_N	102.2(0.0-221.4)	40.7(0.0-118.7)	< 0.0001	0.63 (0.58-0.69)
* VEGF_{AG-N}	-25.29(-93.4-0.0)	0.0(-35.2-0.0)	0.0002	0.61(0.55-0.67)
* IL-1RA_{AG-N}	173.0(31.2-596)	78.7(-33.7-361.9)	0.0052	0.61(0.54-0.68)
IFN-Γ_N	18.7(9.0-38.3)	5.6(1.1-14.3)	<0.0001	0.75(0.71-0.80)
IFN-Γ_{AG-N}	140.3(27.6-353.8)	23.1(0.75-169.4)	<0.0001	0.67(0.62-0.73)
IFN-A2_N	35.2(14.0-52.3)	20.0(7.3-39.9)	0.0046	0.61(0.54-0.69)
SCD40L_N	2400(1217-4587)	1344(765.6-2611)	<0.0001	0.64(0.59-0.70)
MIP-1B_N	771.0(379.6-1737)	1289(648.5-2511)	<0.0001	0.62(0.57-0.68)
TGF-α_N	18.1(11.9-30.3)	9.8(4.2-19.1)	<0.0001	0.69(0.64-0.74)
* TGF-α_{AG-N}	1.4(-1.4-7.3)	0.0(-1.8-2.9)	0.0011	0.59(0.54-0.66)
MMP-9_N	828920(491573-1.284X10 ⁶)	508208(298606-868733)	<0.0001	0.68(0.62-0.73)
MMP-9_{AG-N}	44076(-40408-242490)	4119(-84693-113762)	0.0030	0.59(0.53-0.66)
# IL-1A_N	12.5(1.2-29.7)	14.2(3.1-53.1)	0.0975	0.55(0.49-0.61)

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87 **References**

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- 89 1 Chegou, N. N., Black, G. F., Kidd, M., van Helden, P. D. & Walzl, G. Host markers in
90 QuantiFERON supernatants differentiate active TB from latent TB infection:
91 preliminary report. *BMC pulmonary medicine* **9**, 21, doi:10.1186/1471-2466-9-21
92 (2009).

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