DO COVID ANTIBODIES CROSS-REACT WITH THE BIOPLEX 2200 HIV AG-AB DIFFERENTIATING COMBINATION SCREENING ASSAY?

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BACKGROUND

- Since the COVID 19 virus surge, the dynamics of testing and interpretation have changed
- Multiple COVID 19 tests, and vaccines developed in a small amount of time
- As much is not known and we are still learning about the virus, it is important that we interpret all test results, while keeping COVID in mind
- During August-October 2021, we encountered four repeatedly false-positive samples for HIV at NorthShore University HealthSystem

WHAT WE SAW?

- Four in-house samples were tested Reactive on BioPlex HIV Ag-Ab screening assay
- We ran samples in duplicates on the same lot number of the BioPlex HIV assay, and results were concordant with the previous results

- At Northshore core lab
  - HIV screening with the BioPlex 2200 HIV Ag-Ab, multiplex flow immunoassay
  - Qualitative detection and differentiation of the individual analytes HIV-1 P24 antigen, HIV-1 (group M and O) antibodies, and HIV-2 antibodies in human serum and plasma
- Assay results for each HIV analyte reflected as index
  - Where <1.0 is non-reactive and ≥1.0 is Reactive

- System output will be different when HIV-1 Ab and HIV-2 Ab both have index >1.0
  - If HIV-1 Ab index is at least 5-fold the HIV-2 Ab index, system output will be HIV-1 reactive with its index and HIV-2 nonreactive without index
  - If HIV-2 Ab index is at least 5-fold the HIV-1 Ab index, system output will be HIV-2 reactive with its index and HIV-1 nonreactive without index
  - If the HIV-1 or HIV-2 Ab index is less than 5-fold difference, the output will be reactive, undifferentiated with indices for HIV-1 and HIV-2 Ab

	BIOPLEX ASSAY	INITIAL RUN (IDX)	DUPLICATE 1 &2 (IDX)	RESULTS
P1	HIV Ag-Ab	5.90	4.34, 4.63	Reactive
	HIV-1 Ab	5.90	4.34, 4.63	**Reactive, undifferentiated**
	HIV-1 Ag	0.05	0.03, 0.07	Non- reactive
	HIV-2 Ab	3.34	2.81, 2.63	**Reactive, undifferentiated**
P2	HIV Ag-Ab	33.79	36.76, 39.71	Reactive
	HIV-1 Ab	10.74	10.92, 11.68	**Reactive, undifferentiated**
	HIV-1 Ag	33.79	36.76, 39.71	**Reactive**
	HIV-2 Ab	8.22	8.25, 8.86	**Reactive, undifferentiated**
P3	HIV Ag-Ab	14.80	14.05, 14.30	Reactive
	HIV-1 Ab	3.22	3.40, 3.24	**Reactive, undifferentiated**
	HIV-1 Ag	14.80	14.05, 14.30	**Reactive**
	HIV-2 Ab	2.85	2.55, 2.47	**Reactive, undifferentiated**
P4	HIV Ag-Ab	9.75	10.85, 10.90	Reactive
	HIV-1 Ab	2.40	2.85, 2.40	**Reactive, undifferentiated**
	HIV-1 Ag	9.75	10.85, 10.90	**Reactive**
	HIV-2 Ab	2.14	2.14, 2.13	**Reactive, undifferentiated**

	P24,P31, GP36, GP41,GP140, GP160	GEENIUS RESULT	NAT
P1	Absent	HIV-1 NR HIV-2 NR	Negative
P2	Absent	HIV-1 NR HIV-2 NR	Negative
P3	Absent	HIV-1 NR HIV-2 NR	Negative
P4	Absent	HIV-1 NR HIV-2 NR	Negative

- The samples were tested on Bio-Rad Geenius HIV 1/2 and NAT, indicating false positive

- All four patients had diverse histories except they all had history of COVID vaccination (by Pfizer)

AIM

- To determine the cause of false positivity for our four in-house samples
- Evaluate the potential role of COVID infection and/or vaccination antibodies in the false reactivity of those samples

METHODS AND RESULTS

- Four In-house samples were deidentified, and sent to Bio-Rad Laboratories for further workup
  - Samples were tested on BioPlex HIV (lot 301256, 301227, 301330) and Geenius HIV at Bio-Rad Laboratories
  - Results for BioPlex HIV and Geenius HIV were found to be concordant with in-house results, indicating false positive result for those four in-house samples
- Three cohorts were evaluated for covid antibodies cross-reactivity with HIV screening assay
- All 3 cohort samples were received in frozen state and thawed before testing

COHORT 1

- Cohort One included 121 naturally infected COVID patient samples
- This cohort was used to evaluate COVID antibodies cross reactivity with HIV screening assay in naturally infected patients
  - Samples were acquired from external vendor
  - Patient age ranged from 29 to 77 years and they were collected between 21-133 days after the symptoms onset
  - Samples were evaluated on BioPlex HIV Ag-Ab (lot 301256) and the positive ones were confirmed on Geenius HIV at Bio-Rad Laboratories

- 2/121 naturally infected patient samples tested reactive for HIV-1 antibody on BioPlex HIV assay and both samples confirmed positive on Geenius HIV, Indicating both samples were true positive

- No apparent cross reactivity found with naturally infected patient samples

COHORT 2

- Cohort two included 91 known COVID vaccinated patient samples
- This cohort was used to evaluate COVID antibodies cross reactivity with HIV screening assay in vaccinated patients
  - Had 34 J&J, 26 Pfizer, and 31 Moderna vaccinated patient samples, acquired from external vendor
  - Patient age ranged from 33 to 89 years and they were collected from 7 to 124 days after vaccination
  - Evaluated on BioPlex HIV Ag-Ab (kit lot 301227) and Bio-Rad Geenius HIV assays at Bio-Rad Laboratories

COHORT 2

- Out of 91 vaccinated patient samples, two samples tested reactive on BioPlex HIV assay
- One sample was from J&J vaccinated and other was from Moderna vaccinated cohort
- Both samples didn't confirm on Geenius HIV assay, indicating false-positive HIV results
- Since only two samples tested false positive for HIV, this indicate there is no apparent cross reactivity with vaccinated patient samples

COHORT 2

VACCINE TYPE	BIOPLEX HIV POSITIVE	ASSAY PARAMETERS	IDX	RESULT	GEENIUS HIV
J&J	1/34	HIV Ag-Ab	1.87	Reactive	NR
		HIV-1 Ab	0.04	Non- Reactive	
		HIV-1 Ag	1.87	**Reactive**	
		HIV-2 Ab	0.02	Non- Reactive	
Moderna	1/31	HIV Ag-Ab	3.44	Reactive	NR
		HIV-1 Ab	3.44	**Reactive, Undifferentiated**	
		HIV-1 Ag	0.03	Non- Reactive	
		HIV-2 Ab	1.75	**Reactive, Undifferentiated**	
Pfizer	0/26	--	--	--	--
Total	2/91				

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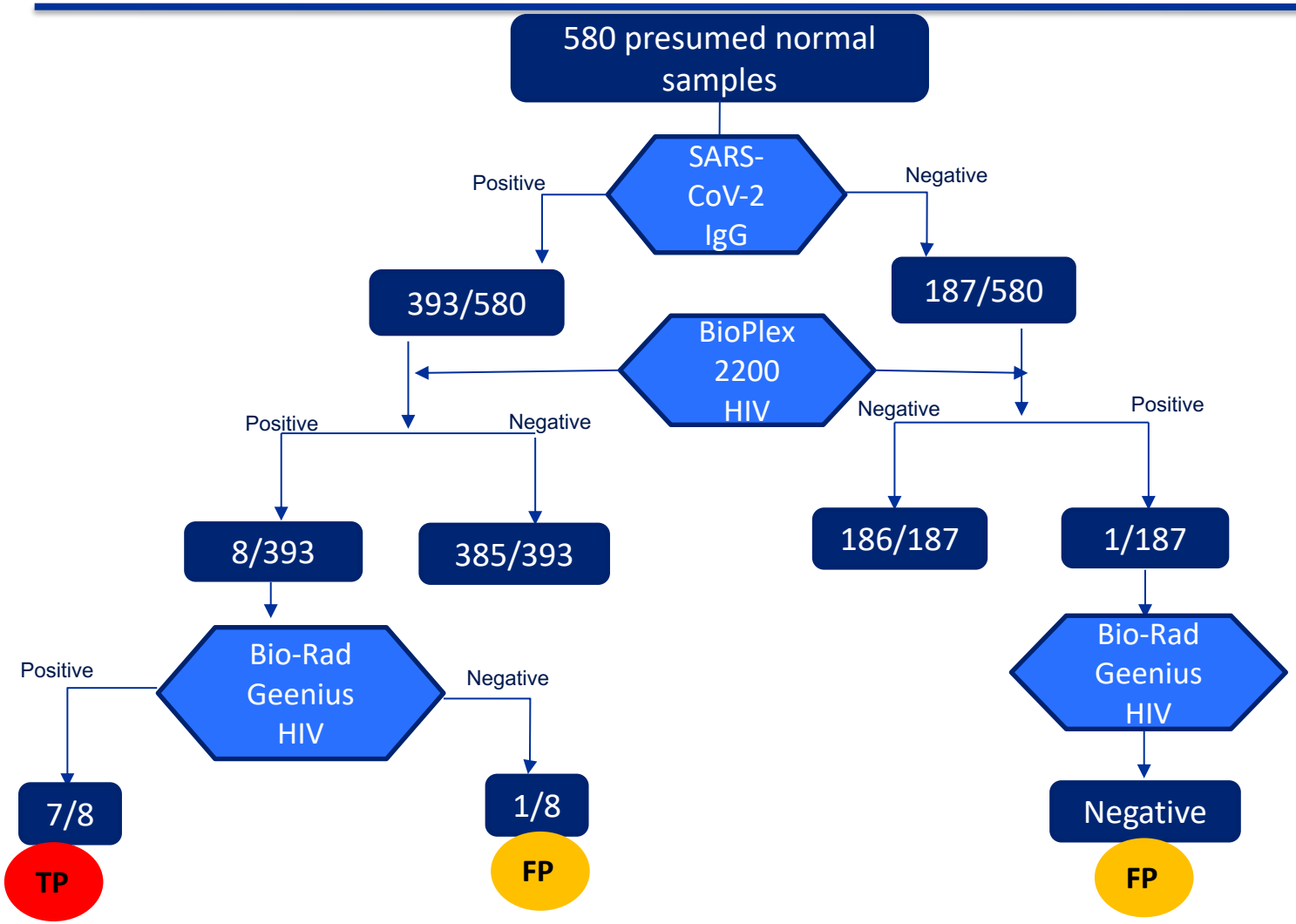
COHORT 3

- Cohort Three included 580 presumed normal patient samples
- This cohort was used to evaluate and compare COVID antibodies cross reactivity with HIV screening assay in COVID positive and negative patient samples
  - Samples were acquired from external vendor after 2019 and patient age ranged from 13 to 97 years
- Samples were evaluated on BioPlex HIV Ag-Ab (kit lot 301330, 301256) and Bio-Rad SARS-CoV-2 IgG assay at Bio-Rad Laboratories
- Fisher's Exact Test used to calculate p-value for sensitivity and specificity of the BioPlex HIV assay in COVID positive and negative cohort and p value <0.05 considered significant

COHORT 3

- 580 presumed normal samples were evaluated on Bio-Rad SARS-CoV-2 IgG assay and 393 samples tested positive
- Nine samples found to be reactive on BioPlex HIV Ag-Ab, seven samples were reactive for HIV-1 antibody and two samples were HIV-Ag reactive
- On further testing on Geenius HIV assay, seven samples confirmed reactive indicating true positive and two HIV-Ag reactive samples did not confirm indicating false positive samples


580 presumed normal samples
SARS-CoV-2 IgG
Positive
Negative
393/580
187/580
BioPlex 2200 HIV
Positive
Negative
Negative
Positive
8/393
385/393
186/187
1/187
Bio-Rad Geenius HIV
Positive
Negative
Bio-Rad Geenius HIV
7/8
TP
1/8
FP
Negative
FP

- Sensitivity and specificity of the BioPlex HIV assay were calculated for both COVID positive and negative groups

- Fisher's Exact Test p-value for sensitivity and specificity was 1.0000 and 0.5466, which was not statistically significant

CONCLUSION

- The results from all three cohorts showed no correlation between COVID antibodies and false-positive HIV screening results
- False-positivity of those four samples were likely due to various residual non-specific immunoassay binding (NSB)
- The Data analysis at our institution reflected BioPlex HIV assay specificity for that time period ranged from 99.87 to 99.89%, which is within the manufacturer's claimed range

THANK YOU