5 May 2020

Pinnacle Biolabs SARS-CoV-2 IgM/IgG Antibody Assay Verification

Extensive verification of the Pinnacle Biolabs COVID-19 Novel Coronavirus IgM/IgG Rapid Test, a lateral flow chromatographic immunoassay, was performed to confirm the specificity and sensitivity of the assay. In particular, verification studies were undertaken to assess:

- Cross-Reactivity/Analytical Specificity
- Analytical Sensitivity
- Clinical Experience

Cross Reactivity/Analytical Specificity

A panel of negative specimens were obtained including 80 serum samples (frozen serum samples stored pre-pandemic), 1 sample (EDTA whole blood) from an individual confirmed to be negative for SARS-CoV-2 via rt-PCR, and 10 fresh fingerstick samples (capillary blood) from individuals confirmed to be negative for SARS-CoV-2 via rt-PCR. All samples were drawn from a population with a high prevalence of vaccination against influenza, HBV, and *Haemophilus influenzae*. The sample were drawn from a population known to contain a high prevalence of HCV. Testing of the samples was performed in accordance with the manufacturer-supplied package insert. Of these samples, 90/91 showed no T1 or T2 bands, indicating a negative result (98.9% overall specificity). 1/91 showed a T2 band only, indicating a negative result for IgM and positive result for IgG (100% specificity for IgM, 98.9% specificity for IgG).

In addition, 5 stored serum samples known to contain anti-RSV IgM and IgG as well as 5 stored serum samples known to contain ANA from individuals were tested. Of these samples, 10/10 showed no T1 or T2 bands, indicating a negative result (100% specificity).

In total, 100/101 samples showed no T1 or T2 bands, indicating a negative result (99% specificity).

Sample	**IgM**	**IgG**	**Comments**
WB-01	Neg	Neg	Negative by rt-PCR
CB-01	Neg	Neg	Negative by rt-PCR
CB-02	Neg	Neg	Negative by rt-PCR
CB-03	Neg	Neg	Negative by rt-PCR
CB-04	Neg	Neg	Negative by rt-PCR
CB-05	Neg	Neg	Negative by rt-PCR
CB-06	Neg	Neg	Negative by rt-PCR
CB-07	Neg	Neg	Negative by rt-PCR
CB-08	Neg	Neg	Negative by rt-PCR
CB-09	Neg	Neg	Negative by rt-PCR
CB-10	Neg	Neg	Negative by rt-PCR
Serum-1	Neg	Neg	

Serum-2	Neg	Neg	
Serum-3	Neg	Neg	
Serum-4	Neg	Neg	
Serum-5	Neg	Neg	
Serum-6	Neg	Neg	
Serum-7	Neg	Neg	
Serum-8	Neg	Neg	
Serum-9	Neg	Neg	
Serum-10	Neg	Neg	
Serum-11	Neg	Neg	
Serum-12	Neg	Neg	
Serum-13	Neg	Neg	
Serum-14	Neg	Neg	
Serum-15	Neg	Neg	
Serum-16	Neg	Neg	
Serum-17	Neg	Neg	
Serum-18	Neg	Neg	
Serum-19	Neg	Pos	Negative for IgG by Abbott Microparticle
Serum-19R	Neg	Neg	
Serum-20	Neg	Neg	
Serum-21	Neg	Neg	
Serum-22	Neg	Neg	
Serum-23	Neg	Neg	
Serum-24	Neg	Neg	
Serum-25	Neg	Neg	
Serum-26	Neg	Neg	
Serum-27	Neg	Neg	
Serum-28	Neg	Neg	
Serum-29	Neg	Neg	
Serum-30	Neg	Neg	
Serum-31	Neg	Neg	
Serum-32	Neg	Neg	
Serum-33	Neg	Neg	

Serum-34	Neg	Neg	
Serum-35	Neg	Neg	
Serum-36	Neg	Neg	
Serum-37	Neg	Neg	
Serum-38	Neg	Neg	
Serum-39	Neg	Neg	
Serum-40	Neg	Neg	
Serum-41	Neg	Neg	
Serum-42	Neg	Neg	
Serum-43	Neg	Neg	
Serum-44	Neg	Neg	
Serum-45	Neg	Neg	
Serum-46	Neg	Neg	
Serum-47	Neg	Neg	
Serum-48	Neg	Neg	
Serum-49	Neg	Neg	
Serum-50	Neg	Neg	
Serum-51	Neg	Neg	
Serum-52	Neg	Neg	
Serum-53	Neg	Neg	
Serum-54	Neg	Neg	
Serum-55	Neg	Neg	
Serum-56	Neg	Neg	
Serum-57	Neg	Neg	
Serum-58	Neg	Neg	
Serum-59	Neg	Neg	
Serum-60	Neg	Neg	
Serum-61	Neg	Neg	
Serum-62	Neg	Neg	
Serum-63	Neg	Neg	
Serum-64	Neg	Neg	
Serum-65	Neg	Neg	
Serum-66	Neg	Neg	

Serum-67	Neg	Neg	
Serum-68	Neg	Neg	
Serum-69	Neg	Neg	
Serum-70	Neg	Neg	
Serum-71	Neg	Neg	
Serum-72	Neg	Neg	
Serum-73	Neg	Neg	
Serum-74	Neg	Neg	
Serum-75	Neg	Neg	
Serum-76	Neg	Neg	
Serum-77	Neg	Neg	
Serum-78	Neg	Neg	
Serum-79	Neg	Neg	
Serum-RSV-1	Neg	Neg	
Serum-RSV-2	Neg	Neg	
Serum-RSV-3	Neg	Neg	
Serum-RSV-4	Neg	Neg	
Serum-RSV-5	Neg	Neg	
Serum-ANA-1	Neg	Neg	
Serum-ANA-2	Neg	Neg	
Serum-ANA-3	Neg	Neg	
Serum-ANA-4	Neg	Neg	
Serum-ANA-5	Neg	Neg	

Analytical Sensitivity

A panel of 15 known positive specimens (whole blood, EDTA) were obtained from Christiana Hospital. Immune status of the individuals or length of active infection was not known or collected. Of the 15 known positive specimens, 12 demonstrated a positive IgM and IgG band on the rapid test (80% sensitivity).

Discussion

Consistent with FDA guidance, docket FDA-2020-D-0987, the State of Delaware has identified point-of-care lateral flow immunoassays (“rapid tests”) as useful diagnostic adjuncts for COVID-19 and subsequently developed guidance for use of these tests. Use of rapid tests is contingent upon implementation in appropriate clinical scenarios, leveraging high analytical specificity (>99%) in a “PCR-sparing” testing strategy.

Over a period 4/7/2020-4/29/2020, DPH monitored samples as part of a prospective observational effort to ensure satisfactory performance of Pinnacle Biolabs COVID-19 Novel Coronavirus IgM/IgG Rapid Tests in a real-world setting. IRB approval was not required as testing was performed under executive authority consistent with the Eleventh Modification of the Declaration of a State of Emergency for the State of Delaware Due to a Public Health Threat.

Specimens were deployed by Delaware Division of Public Health personnel, principally within post-acute care facilities. Tests were administered by licensed registered nurses (RNs) or physicians (MD/DOs) following training performed in-person or via instructional video. Specimens were collected in accordance with the manufacturer-supplied package insert. Specimens were collected simultaneously with nasopharyngeal swabs and compared to rt-PCR results.

Of these specimens, high specificity was maintained with no false positives identified by rt-PCR. Most specimens were identified to manifest both IgM and IgG, with some specimens showing IgM only and only a few showing IgG only. Multiple patients known to have remote infection with SARS-CoV-2 via positive rt-PCR testing manifested both IgM and IgG, and were found to have both IgM and IgG. Repeat PCR testing showed threshold cycle values ranging from low (17) to high (34).

Rapid tests may be deployed first in symptomatic individuals and a positive IgM is a reasonable surrogate to identify infection with COVID-19, and follow-up PCR is not necessary, as the sensitivity of widely available PCR and negative predictive value given high-pretest probability with positive serology would not be sufficient to change recommendations for isolation. Those individuals with negative rapid tests should then proceed to molecular testing via PCR.