



Agricultural **BIOTECHNOLOGY** Research Institute

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No.....291.....

Date...09-03-2021.

To

Dr. Tassawar Hussain Malik
Director Research (Headquarters)
Pakistan Central Cotton Committee (PCCC)
Old Shujabad road, Multan - 60500 - Punjab, Pakistan

Subject:- **2020 NCVT BIOCHEMICAL TESTING (BCT) RESULTS**

Vide reference to your letter no. PCCC-DR-675-BCT-2020-469 dated 15-06-2020.

The detailed Biochemical testing report of 110 NCVT entries received from your good office vide letter dated mentioned above are attached herewith for your information and further necessary action.

(DR. SAJID UR RAHMAN)
CHIEF SCIENTIST
AGRI. BIOTECHNOLOGY RES. INSTT
AARI, FAISALABAD

BIOTECH TESTING REPORT OF CANDIDATE COTTON VARIETIES OF NATIONAL COORDINATED VARIETAL TRIALS (NCVT-2020)

Cotton seeds of 110 entries coded as (PC-2001 to PC-2110) of NCVT-2020, received from Directorate of Agricultural Research, Pakistan Central Cotton Committee (PCCC), Multan, vide your letter No. F.PCCC/DR(575)BCT-2020/469 Dated: 15-06-2020 for Biochemical testing were tested at this institute as per SOPs. Details of the desired assays are given below.

1. Purity Test

Procedure:

Entries were tested for presence of *Cry1A*, *Cry2A*, *RR* and *Vip3A* proteins in leaf tissues. Two leaf discs were sampled from 80 days old plants for the assay. Five consecutive plants in a row were tested for presence of the proteins through lateral flow strip test (QuickStix Combo Kit: AS 046 LS for *Cry1A*, *Cry2A* & CP4-EPSPS, ENVIROLOGIX Inc and QuickStix AS-485-LSS for *Vip3A*) following manufacturer's instructions.

2. DNA Test

Procedure:

1. Total genomic DNA of positive entries (one positive plant from each entry), earlier detected through lateral flow strip test, was isolated using CTAB method.
2. Microsatellite marker CIR-183 was amplified from isolated DNA as an internal control to check the quality of DNA.
3. For event detection of MON531, event specific primer Mon531 (genome/cry1Ac, 346bp) was used.
4. Entries found positive for *Cry2A*, *RR* and *Vip3A* were tested for MON15985, MON1445 & COT102 events using protocol developed by Monsanto Company and validated by European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), Biotechnology and GMOs unit- Community Reference Lab for GM food and Feed, Italy.
5. DNA from known source of cotton containing MON531 event was used as positive control. One reaction with DNA of non-Bt cotton variety and other reaction with d_3H_2O were added as negative controls.

6. Amplification was performed in Mastercycler gradient; (Eppendorf, Germany), products were resolved on 2% agarose gel and stained with ethidium bromide.

Quantitative ELISA

Procedure:

One plant from each entry, found positive for Cry1Ac through lateral flow strip test, was tested for quantification of Cry1Ac endotoxins at 80 days after sowing. A youngest fully expanded, main stem leaf (18 days old) was used for quantitative ELISA. The assay was performed following manufacturer's instructions. Procedure was conducted on μ quant BioTek micro plate reader and GEN5 software was used for analysis.

Note: Protein detection assay is highly sensitive and may be influenced by several biotic and abiotic factors. Expression is highly dependent on the age of leaf. Moreover, detection kits from different manufacturers may yield variable results hence; results among the Labs may vary.

Shankar

Results of Biochemical Tests-2020

Name of Laboratory: GMO testing lab, Agricultural Biotechnology Research Institute,
AARI, Faisalabad

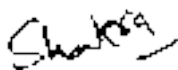
Gene/Event/Trait under Test: Test results of the gene Cry1Ac (Event MON-531), Cry2Ab
(Event MON-15985), RR (Event MON-1445) and Vip3A
(Event COT102)

Sr. No.	Sample code	Immunostrip Analysis							Toxin Expression (µg/g of fresh leaves 80 DAP)
		Trait Confirmation (+/-)				Trait Purity (%)			
		Cry 1Ac	Cry 2Ab	RR	Vip3A	Cry 1Ac	Cry 2Ab	RR	
1	PC-2001	-ve				0	0	0	0
2	PC-2002	+ve	-ve	-ve	-ve	100	0	0	1.24
3	PC-2003	-ve				0	0	0	0
4	PC-2004	-ve				0	0	0	0
5	PC-2005	-ve				0	0	0	0
6	PC-2006	+ve	-ve	-ve	-ve	80	0	0	0.96
7	PC-2007	-ve				0	0	0	0
8	PC-2008	+ve	-ve	-ve	-ve	40	0	0	0.20
9	PC-2009	+ve	-ve	-ve	-ve	100	0	0	2.00
10	PC-2010	+ve	-ve	-ve	-ve	100	0	0	4.22
11	PC-2011	+ve	-ve	+ve	-ve	100	0	100	2.8
12	PC-2012	+ve	-ve	+ve	-ve	100	0	80	1.24
13	PC-2013	+ve	-ve	-ve	-ve	100	0	0	2.00
14	PC-2014	+ve	-ve	-ve	-ve	100	0	0	1.42
15	PC-2015	+ve	-ve	-ve	-ve	100	0	0	0.86
16	PC-2016	+ve	-ve	-ve	-ve	100	0	40	5.68
17	PC-2017	+ve	-ve	+ve	-ve	100	0	80	1.39
18	PC-2018	+ve	-ve	-ve	-ve	100	0	0	0.92
19	PC-2019	+ve	-ve	-ve	-ve	100	0	0	3.24
20	PC-2020	+ve	-ve	-ve	-ve	100	0	0	1.64
21	PC-2021	+ve	-ve	-ve	-ve	80	0	0	1.12
22	PC-2022	+ve	-ve	-ve	-ve	100	0	0	2.31
23	PC-2023	+ve	-ve	-ve	-ve	100	0	0	1.54
24	PC-2024	-ve				0	0	0	0
25	PC-2025	+ve	+ve	+ve	-ve	100	40	60	1.08
26	PC-2026	+ve	-ve	-ve	-ve	100	0	0	1.25
27	PC-2027	+ve	-ve	-ve	-ve	100	0	0	1.26

28	PC-2028	+ve	-ve	-ve	-ve	100	0	0	2.32
29	PC-2029	+ve	-ve	-ve	-ve	100	0	0	3.00
30	PC-2030	+ve	-ve	-ve	-ve	100	0	0	2.12
31	PC-2031	+ve	+ve	+ve	-ve	100	100	100	3.24
32	PC-2032	+ve	-ve	-ve	-ve	100	0	0	4.32
33	PC-2033	+ve	-ve	+ve	-ve	100	0	100	1.36
34	PC-2034	+ve	-ve	-ve	-ve	100	0	0	2.21
35	PC-2035	+ve	-ve	-ve	-ve	100	0	0	1.63
36	PC-2036	+ve	-ve	-ve	-ve	100	0	0	3.24
37	PC-2037	+ve	-ve	-ve	-ve	100	0	0	1.34
38	PC-2038	+ve	-ve	-ve	-ve	100	0	0	2.81
39	PC-2039	+ve	-ve	-ve	-ve	100	0	0	3.80
40	PC-2040	+ve	-ve	-ve	-ve	100	0	0	2.45
41	PC-2041	+ve	-ve	-ve	-ve	100	0	0	0.76
42	PC-2042	+ve	-ve	+ve	-ve	100	0	100	2.45
43	PC-2043	+ve	-ve	-ve	-ve	100	0	0	1.52
44	PC-2044	+ve	-ve	+ve	-ve	100	0	100	3.96
45	PC-2045	+ve	-ve	-ve	-ve	100	0	0	1.48
46	PC-2046	+ve	-ve	-ve	-ve	100	0	0	2.6
47	PC-2047	+ve	-ve	-ve	-ve	100	0	0	3.34
48	PC-2048	+ve	-ve	-ve	-ve	100	0	0	2.52
49	PC-2049	+ve	-ve	+ve	-ve	100	0	60	2.87
50	PC-2050	+ve	-ve	-ve	-ve	100	0	0	1.72
51	PC-2051	+ve	-ve	+ve	-ve	60	0	100	1.29
52	PC-2052	+ve	-ve	-ve	-ve	80	0	0	2.62
53	PC-2053	+ve	-ve	-ve	-ve	100	0	100	3.44
54	PC-2054	+ve	+ve	+ve	-ve	100	100	100	2.30
55	PC-2055	+ve	-ve	-ve	-ve	100	0	0	1.76
56	PC-2056	+ve	-ve	-ve	-ve	40	0	0	1.55
57	PC-2057	+ve	-ve	-ve	-ve	100	0	0	0.39
58	PC-2058	+ve	-ve	+ve	-ve	100	0	80	5.30
59	PC-2059	+ve	-ve	-ve	-ve	100	0	0	5.79
60	PC-2060	+ve	-ve	-ve	-ve	100	0	0	1.68
61	PC-2061	+ve	-ve	-ve	-ve	100	0	0	1.08
62	PC-2062	+ve	-ve	+ve	-ve	100	0	100	2.88
63	PC-2063	+ve	-ve	+ve	-ve	100	0	100	1.38
64	PC-2064	+ve	-ve	+ve	-ve	100	0	40	3.18
65	PC-2065	+ve	-ve	-ve	-ve	100	0	0	5.30

66	PC-2066	+ve	-ve	-ve	-ve	100	0	0	3.16
67	PC-2067	+ve	-ve	+ve	-ve	100	0	40	5.96
68	PC-2068	+ve	-ve	-ve	-ve	100	0	0	3.11
69	PC-2069	+ve	-ve	-ve	-ve	100	0	0	3.49
70	PC-2070	+ve	-ve	-ve	-ve	100	0	0	1.65
71	PC-2071	+ve	-ve	+ve	-ve	100	0	100	4.76
72	PC-2072	+ve	-ve	-ve	-ve	100	0	0	2.69
73	PC-2073	+ve	-ve	-ve	-ve	100	0	0	2.81
74	PC-2074	+ve	-ve	-ve	-ve	100	0	0	2.83
75	PC-2075	+ve	-ve	+ve	-ve	100	0	100	1.42
76	PC-2076	+ve	-ve	-ve	-ve	100	0	0	2.02
77	PC-2077	+ve	-ve	-ve	-ve	100	0	0	3.64
78	PC-2078	+ve	-ve	-ve	-ve	100	0	0	1.23
79	PC-2079	+ve	-ve	-ve	-ve	100	0	0	2.63
80	PC-2080	-ve				0	0	0	1.33
81	PC-2081	+ve	-ve	+ve	-ve	100	0	100	3.84
82	PC-2082	+ve	-ve	-ve	-ve	100	0	0	1.03
83	PC-2083	+ve	-ve	-ve	-ve	100	0	0	1.56
84	PC-2084	+ve	-ve	-ve	-ve	100	0	0	2.51
85	PC-2085	+ve	-ve	-ve	-ve	100	0	0	3.23
86	PC-2086	+ve	-ve	+ve	-ve	100	0	100	2.73
87	PC-2087	-ve				0	0	0	0
88	PC-2088	-ve				0	0	0	0
89	PC-2089	+ve	-ve	-ve	-ve	100	0	0	1.64
90	PC-2090	+ve	-ve	-ve	-ve	100	0	0	0.81
91	PC-2091	+ve	-ve	-ve	-ve	100	0	0	5.20
92	PC-2092	-ve				0	0	0	0
93	PC-2093	+ve	-ve	-ve	-ve	80	0	0	3.01
94	PC-2094	+ve	-ve	-ve	-ve	60	0	0	1.21
95	PC-2095	-ve				0	0	0	0
96	PC-2096	-ve				0	0	0	0
97	PC-2097	+ve	-ve	-ve	-ve	80	0	0	2.80
98	PC-2098	-ve				0	0	0	0
99	PC-2099	-ve				0	0	0	0
100	PC-2100	+ve	-ve	-ve	-ve	100	0	0	2.29
101	PC-2101	+ve	-ve	-ve	-ve	100	0	0	2.11
102	PC-2102	+ve	-ve	-ve	-ve	100	0	0	2.81
103	PC-2103	+ve	-ve	-ve	-ve	100	0	0	4.42

104	PC-2104	+ve	-ve	-ve	-ve	100	0	0	3.46
105	PC-2105	+ve	-ve	-ve	-ve	100	0	0	2.69
106	PC-2106	+ve	-ve	+ve	-ve	100	0	100	1.95
107	PC-2107	+ve	-ve	+ve	-ve	100	0	100	2.84
108	PC-2108	+ve	+ve	+ve	-ve	100	100	100	1.24
109	PC-2109	+ve	-ve	+ve	-ve	100	0	100	3.29
110	PC-2110	+ve	-ve	+ve	-ve	100	0	100	1.76



Laboratory Incharge:
Shakra Jamil, Botanist Biotechnology,
Agricultural Biotechnology Research
Institute, Faisalabad



Director of the Institute:
Dr. Sajid-Ur-Rahman
Agricultural Biotechnology Research
Institute, Faisalabad

Performa Sheet for submission of results of Biochemical Tests-2020

a- **Name of Laboratory:** CEMB University of the Punjab Lahore

b- **Gene/Event/Trait under Test:** CryIAc

Sr.	Sample Code (BCT17)	Immunostrip Analysis		PCR Confirmation/ Analysis		Toxin Expression (µg/g of fresh leaves 80DAP)		Remarks
		Trait Confirmation (+/-)	Trait Purity (%)	Trait Confirmation (+/-)	Trait Purity (%)	ELISA Antigen, Antibody Reaction	PCR/ qPCR	
1.	PC-2001	+ve	100	+ve	100	2.921	+ve	
2.	PC-2002	+ve	100	+ve	100	2.803	+ve	
3.	PC-2003	+ve	100	+ve	100	2.532	+ve	
4.	PC-2004	+ve	100	+ve	100	2.654	+ve	
5.	PC-2005	-ve	0	-ve	0	-ve	-ve	
6.	PC-2006	+ve	100	+ve	100	2.376	+ve	
7.	PC-2007	+ve	100	+ve	100	2.703	+ve	
8.	PC-2008	+ve	100	+ve	100	3.634	+ve	
9.	PC-2009	+ve	100	+ve	100	3.236	+ve	
10.	PC-2010	+ve	100	+ve	100	3.653	+ve	
11.	PC-2011	+ve	100	+ve	100	3.251	+ve	
12.	PC-2012	+ve	100	+ve	100	3.360	+ve	
13.	PC-2013	+ve	100	+ve	100	3.253	+ve	
14.	PC-2014	+ve	100	+ve	100	3.691	+ve	
15.	PC-2015	+ve	100	+ve	100	2.921	+ve	
16.	PC-2016	+ve	100	+ve	100	2.803	+ve	
17.	PC-2017	+ve	100	+ve	100	2.532	+ve	
18.	PC-2018	+ve	100	+ve	100	2.654	+ve	
19.	PC-2019	+ve	100	+ve	100	3.982	+ve	
20.	PC-2020	+ve	100	+ve	100	3.053	+ve	
21.	PC-2021	+ve	100	+ve	100	3.762	+ve	
22.	PC-2022	+ve	100	+ve	100	3.846	+ve	
23.	PC-2023	+ve	100	+ve	100	3.564	+ve	
24.	PC-2024	+ve	100	+ve	100	3.651	+ve	
25.	PC-2025	+ve	100	+ve	100	3.064	+ve	
26.	PC-2026	+ve	100	+ve	100	3.521	+ve	
27.	PC-2027	+ve	100	+ve	100	3.351	+ve	
28.	PC-2028	+ve	100	+ve	100	3.253	+ve	
29.	PC-2029	+ve	100	+ve	100	2.985	+ve	
30.	PC-2030	+ve	100	+ve	100	3.615	+ve	
31.	PC-2031	+ve	100	+ve	100	3.518	+ve	
32.	PC-2032	-ve	0	-ve	0	-ve	-ve	
33.	PC-2033	+ve	100	+ve	100	3.301	+ve	
34.	PC-2034	+ve	100	+ve	100	2.620	+ve	
35.	PC-2035	+ve	100	+ve	100	3.251	+ve	
36.	PC-2036	+ve	100	+ve	100	2.360	+ve	
37.	PC-2037	+ve	100	+ve	100	3.650	+ve	
38.	PC-2038	+ve	100	+ve	100	2.741	+ve	
39.	PC-2039	+ve	100	+ve	100	2.505	+ve	

40.	PC-2040	+ve	100	+ve	100	2.052	+ve
41.	PC-2041	+ve	100	+ve	100	2.800	+ve
42.	PC-2042	+ve	100	+ve	100	3.192	+ve
43.	PC-2043	+ve	100	+ve	100	2.920	+ve
44.	PC-2044	+ve	100	+ve	100	2.977	+ve
45.	PC-2045	+ve	100	+ve	100	2.153	+ve
46.	PC-2046	+ve	100	+ve	100	3.170	+ve
47.	PC-2047	-ve	0	-ve	0	-ve	-ve
48.	PC-2048	+ve	100	+ve	100	2.341	+ve
49.	PC-2049	+ve	100	+ve	100	2.405	+ve
50.	PC-2050	+ve	100	+ve	100	2.252	+ve
51.	PC-2051	+ve	100	+ve	100	2.600	+ve
52.	PC-2052	+ve	100	+ve	100	3.192	+ve
53.	PC-2053	+ve	100	+ve	100	3.388	+ve
54.	PC-2054	+ve	100	+ve	100	3.482	+ve
55.	PC-2055	+ve	100	+ve	100	3.310	+ve
56.	PC-2056	+ve	100	+ve	100	2.390	+ve
57.	PC-2057	+ve	100	+ve	100	3.621	+ve
58.	PC-2058	+ve	100	+ve	100	2.004	+ve
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60.	PC-2060	+ve	100	+ve	100	2.662	+ve
61.	PC-2061	+ve	100	+ve	100	2.465	+ve
62.	PC-2062	+ve	100	+ve	100	2.502	+ve
63.	PC-2063	+ve	100	+ve	100	3.688	+ve
64.	PC-2064	+ve	100	+ve	100	3.793	+ve
65.	PC-2065	+ve	100	+ve	100	2.626	+ve
66.	PC-2066	+ve	100	+ve	100	2.477	+ve
67.	PC-2067	+ve	100	+ve	100	3.288	+ve
68.	PC-2068	-ve	0	-ve	0	-ve	-ve
69.	PC-2069	+ve	100	+ve	100	2.532	+ve
70.	PC-2070	+ve	100	+ve	100	3.212	+ve
71.	PC-2071	+ve	100	+ve	100	3.320	+ve
72.	PC-2072	+ve	100	+ve	100	2.290	+ve
73.	PC-2073	+ve	100	+ve	100	3.421	+ve
74.	PC-2074	+ve	100	+ve	100	2.304	+ve
75.	PC-2075	+ve	100	+ve	100	2.351	+ve
76.	PC-2076	+ve	100	+ve	100	2.362	+ve
77.	PC-2077	+ve	100	+ve	100	2.665	+ve
78.	PC-2078	+ve	100	+ve	100	2.302	+ve
79.	PC-2079	+ve	100	+ve	100	3.687	+ve
80.	PC-2080	+ve	100	+ve	100	2.721	+ve
81.	PC-2081	+ve	100	+ve	100	2.497	+ve
82.	PC-2082	+ve	100	+ve	100	3.277	+ve
83.	PC-2083	+ve	100	+ve	100	2.203	+ve
84.	PC-2084	+ve	100	+ve	100	3.850	+ve
85.	PC-2085	+ve	100	+ve	100	3.212	+ve
86.	PC-2086	+ve	100	+ve	100	3.490	+ve

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87.	PC-2087	+ve	100	+ve	100	3.690	+ve	
88.	PC-2088	+ve	100	+ve	100	3.470	+ve	
89.	PC-2089	+ve	100	+ve	100	3.316	+ve	
90.	PC-2090	+ve	100	+ve	100	2.621	+ve	
91.	PC-2091	+ve	100	+ve	100	3.260	+ve	
92.	PC-2092	+ve	100	+ve	100	3.369	+ve	
93.	PC-2093	+ve	100	+ve	100	2.510	+ve	
94.	PC-2094	+ve	100	+ve	100	2.212	+ve	
95.	PC-2095	+ve	100	+ve	100	3.313	+ve	
96.	PC-2096	+ve	100	+ve	100	2.451	+ve	
97.	PC-2097	+ve	100	+ve	100	3.232	+ve	
98.	PC-2098	+ve	100	+ve	100	2.615	+ve	
99.	PC-2099	+ve	100	+ve	100	3.432	+ve	
100.	PC-20100	+ve	100	+ve	100	3.221	+ve	
101.	PC-20101	+ve	100	+ve	100	2.643	+ve	
102.	PC-20102	+ve	100	+ve	100	3.420	+ve	
103.	PC-20103	+ve	100	+ve	100	3.531	+ve	
104.	PC-20104	+ve	100	+ve	100	2.213	+ve	
105.	PC-20105	+ve	100	+ve	100	3.501	+ve	
106.	PC-20106	+ve	100	+ve	100	3.662	+ve	
107.	PC-20107	+ve	100	+ve	100	3.615	+ve	
108.	PC-20108	+ve	100	+ve	100	3.802	+ve	
109.	PC-20109	+ve	100	+ve	100	3.442	+ve	
110.	PC-20110	+ve	100	+ve	100	3.325	+ve	



Done by



Checked by



Verified by

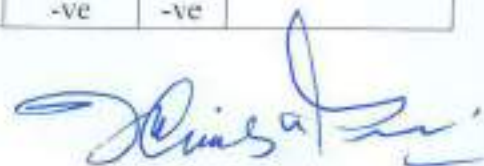
Performa Sheet for submission of results of Biochemical Tests-2020

a- **Name of Laboratory:** CEMB University of the Punjab Lahore

b- **Gene/Event/Trait under Test:** Cry2A

Sr.	Sample Code (BCF17)	Immunostrip Analysis		PCR Confirmation/ Analysis		Toxin Expression (µg/g of fresh leaves 80DAP)		Remarks
		Trait Confirmation (+/-)	Trait Purity (%)	Trait Confirmation (+/-)	Trait Purity (%)	ELISA Antigen, Antibody Reaction	PCR/ qPCR	
1.	PC-2001	-ve	0	-ve	0	-ve	-ve	
2.	PC-2002	-ve	0	-ve	0	-ve	-ve	
3.	PC-2003	-ve	0	-ve	0	-ve	-ve	
4.	PC-2004	-ve	0	-ve	0	-ve	-ve	
5.	PC-2005	-ve	0	-ve	0	-ve	-ve	
6.	PC-2006	-ve	0	-ve	0	-ve	-ve	
7.	PC-2007	-ve	0	-ve	0	-ve	-ve	
8.	PC-2008	-ve	0	+ve	0	-ve	-ve	
9.	PC-2009	-ve	0	-ve	0	-ve	-ve	
10.	PC-2010	-ve	0	-ve	0	-ve	-ve	
11.	PC-2011	+ve	100	+ve	100	2.765	+ve	
12.	PC-2012	+ve	100	+ve	100	2.653	+ve	
13.	PC-2013	-ve	0	-ve	0	-ve	-ve	
14.	PC-2014	-ve	0	-ve	0	-ve	-ve	
15.	PC-2015	-ve	0	-ve	0	-ve	-ve	
16.	PC-2016	-ve	0	-ve	0	-ve	-ve	
17.	PC-2017	+ve	100	+ve	100	2.532	+ve	
18.	PC-2018	+ve	100	+ve	100	2.654	+ve	
19.	PC-2019	-ve	0	-ve	0	-ve	-ve	
20.	PC-2020	-ve	0	-ve	0	-ve	-ve	
21.	PC-2021	-ve	0	-ve	0	-ve	-ve	
22.	PC-2022	-ve	0	-ve	0	-ve	-ve	
23.	PC-2023	-ve	0	-ve	0	-ve	-ve	
24.	PC-2024	-ve	0	-ve	0	-ve	-ve	
25.	PC-2025	+ve	100	+ve	100	2.324	+ve	
26.	PC-2026	-ve	0	-ve	0	-ve	-ve	
27.	PC-2027	+ve	100	+ve	100	2.442	+ve	
28.	PC-2028	+ve	100	+ve	100	2.643	+ve	
29.	PC-2029	+ve	100	+ve	100	2.335	+ve	
30.	PC-2030	+ve	100	+ve	100	2.453	+ve	
31.	PC-2031	+ve	100	+ve	100	2.432	+ve	
32.	PC-2032	-ve	0	-ve	0	-ve	-ve	
33.	PC-2033	+ve	100	+ve	100	2.920	+ve	
34.	PC-2034	-ve	0	-ve	0	-ve	-ve	
35.	PC-2035	+ve	100	+ve	100	3.256	+ve	
36.	PC-2036	-ve	0	-ve	0	-ve	-ve	
37.	PC-2037	-ve	0	-ve	0	-ve	-ve	
38.	PC-2038	-ve	0	-ve	0	-ve	-ve	
39.	PC-2039	-ve	0	-ve	0	-ve	-ve	





40.	PC-2040	-ve	0	+ve	0	-ve	-ve	
41.	PC-2041	-ve	0	-ve	0	-ve	-ve	
42.	PC-2042	+ve	100	+ve	100	2.432	+ve	
43.	PC-2043	+ve	100	+ve	100	2.320	+ve	
44.	PC-2044	+ve	100	+ve	100	2.332	+ve	
45.	PC-2045	+ve	100	+ve	100	2.153	+ve	
46.	PC-2046	-ve	0	-ve	0	-ve	-ve	
47.	PC-2047	-ve	0	-ve	0	-ve	-ve	
48.	PC-2048	+ve	100	+ve	100	2.662	+ve	
49.	PC-2049	+ve	100	+ve	100	2.434	+ve	
50.	PC-2050	-ve	0	-ve	0	-ve	-ve	
51.	PC-2051	+ve	100	+ve	100	2.552	+ve	
52.	PC-2052	-ve	0	-ve	0	-ve	-ve	
53.	PC-2053	+ve	100	+ve	100	3.434	+ve	
54.	PC-2054	+ve	100	+ve	100	2.482	+ve	
55.	PC-2055	-ve	0	-ve	0	-ve	-ve	
56.	PC-2056	-ve	0	-ve	0	-ve	-ve	
57.	PC-2057	-ve	0	-ve	0	-ve	-ve	
58.	PC-2058	+ve	100	+ve	100	2.525	+ve	
59.	PC-2059	+ve	100	+ve	100	2.444	+ve	
60.	PC-2060	+ve	100	+ve	100	2.690	+ve	
61.	PC-2061	-ve	0	-ve	0	-ve	-ve	
62.	PC-2062	+ve	100	+ve	100	2.332	+ve	
63.	PC-2063	+ve	100	+ve	100	2.423	+ve	
64.	PC-2064	-ve	0	-ve	0	-ve	-ve	
65.	PC-2065	-ve	0	-ve	0	-ve	-ve	
66.	PC-2066	-ve	0	-ve	0	-ve	-ve	
67.	PC-2067	+ve	100	+ve	100	3.288	+ve	
68.	PC-2068	-ve	0	-ve	0	-ve	-ve	
69.	PC-2069	-ve	0	-ve	0	-ve	-ve	
70.	PC-2070	-ve	0	-ve	0	-ve	-ve	
71.	PC-2071	+ve	100	+ve	100	2.310	+ve	
72.	PC-2072	-ve	0	-ve	0	-ve	-ve	
73.	PC-2073	-ve	0	-ve	0	-ve	-ve	
74.	PC-2074	-ve	0	-ve	0	-ve	-ve	
75.	PC-2075	+ve	100	+ve	100	2.451	+ve	
76.	PC-2076	-ve	0	-ve	0	-ve	-ve	
77.	PC-2077	-ve	0	-ve	0	-ve	-ve	
78.	PC-2078	+ve	100	+ve	100	3.502	+ve	
79.	PC-2079	+ve	100	+ve	100	2.652	+ve	
80.	PC-2080	-ve	0	-ve	0	-ve	-ve	
81.	PC-2081	+ve	100	+ve	100	2.497	+ve	
82.	PC-2082	-ve	0	-ve	0	-ve	-ve	
83.	PC-2083	+ve	100	+ve	100	2.762	+ve	
84.	PC-2084	+ve	100	+ve	100	2.750	+ve	
85.	PC-2085	+ve	100	+ve	100	2.432	+ve	
86.	PC-2086	+ve	100	+ve	100	2.590	+ve	

87.	PC-2087	-ve	0	-ve	0	-ve	-ve	
88.	PC-2088	-ve	0	-ve	0	-ve	-ve	
89.	PC-2089	+ve	100	+ve	100	2.616	+ve	
90.	PC-2090	+ve	100	+ve	100	2.421	+ve	
91.	PC-2091	+ve	100	+ve	100	2.623	+ve	
92.	PC-2092	-ve	0	-ve	0	-ve	-ve	
93.	PC-2093	+ve	100	+ve	100	2.453	+ve	
94.	PC-2094	-ve	0	-ve	0	-ve	-ve	
95.	PC-2095	+ve	100	+ve	100	2.562	+ve	
96.	PC-2096	+ve	100	+ve	100	2.332	+ve	
97.	PC-2097	-ve	0	-ve	0	-ve	-ve	
98.	PC-2098	+ve	100	+ve	100	2.442	+ve	
99.	PC-2099	+ve	100	+ve	100	2.132	+ve	
100.	PC-20100	-ve	0	-ve	0	-ve	-ve	
101.	PC-20101	-ve	0	-ve	0	-ve	-ve	
102.	PC-20102	-ve	0	-ve	0	-ve	-ve	
103.	PC-20103	+ve	100	+ve	100	2.431	+ve	
104.	PC-20104	-ve	0	-ve	0	-ve	-ve	
105.	PC-20105	-ve	0	-ve	0	-ve	-ve	
106.	PC-20106	+ve	100	+ve	100	2.422	+ve	
107.	PC-20107	+ve	100	+ve	100	2.025	+ve	
108.	PC-20108	+ve	100	+ve	100	2.502	+ve	
109.	PC-20109	+ve	100	+ve	100	2.352	+ve	
110.	PC-20110	+ve	100	+ve	100	2.425	+ve	

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Performa Sheet for submission of results of Biochemical Tests-2020

a- Name of Laboratory: CEMB University of the Punjab Lahore

b- Gene/Event/Trait under Test: CEMB-GTG

Sr.	Sample Code (BCF17)	Immunostrip Analysis		PCR Confirmation/ Analysis		Toxin Expression ($\mu\text{g/g}$ of fresh leaves 80DAP)		Remarks
		Trait Confirmation (+/-)	Trait Purity (%)	Trait Confirmation (+/-)	Trait Purity (%)	ELISA Antigen, Antibody Reaction	PCR/ qPCR	
1.	PC-2001	-ve	0	-ve	0	-ve	-ve	
2.	PC-2002	-ve	0	-ve	0	-ve	-ve	
3.	PC-2003	-ve	0	-ve	0	-ve	-ve	
4.	PC-2004	-ve	0	-ve	0	-ve	-ve	
5.	PC-2005	-ve	0	-ve	0	-ve	-ve	
6.	PC-2006	-ve	0	-ve	0	-ve	-ve	
7.	PC-2007	-ve	0	-ve	0	-ve	-ve	
8.	PC-2008	-ve	0	+ve	0	-ve	-ve	
9.	PC-2009	-ve	0	-ve	0	-ve	-ve	
10.	PC-2010	-ve	0	-ve	0	-ve	-ve	
11.	PC-2011	+ve	100	+ve	100	2.465	+ve	
12.	PC-2012	-ve	0	-ve	0	-ve	-ve	
13.	PC-2013	-ve	0	-ve	0	-ve	-ve	
14.	PC-2014	-ve	0	-ve	0	-ve	-ve	
15.	PC-2015	-ve	0	-ve	0	-ve	-ve	
16.	PC-2016	-ve	0	-ve	0	-ve	-ve	
17.	PC-2017	-ve	0	-ve	0	-ve	-ve	
18.	PC-2018	+ve	100	+ve	100	2.354	+ve	
19.	PC-2019	-ve	0	-ve	0	-ve	-ve	
20.	PC-2020	-ve	0	-ve	0	-ve	-ve	
21.	PC-2021	-ve	0	-ve	0	-ve	-ve	
22.	PC-2022	-ve	0	-ve	0	-ve	-ve	
23.	PC-2023	-ve	0	-ve	0	-ve	-ve	
24.	PC-2024	-ve	0	-ve	0	-ve	-ve	
25.	PC-2025	+ve	100	+ve	100	2.623	+ve	
26.	PC-2026	-ve	0	-ve	0	-ve	-ve	
27.	PC-2027	+ve	100	+ve	100	2.342	-ve	
28.	PC-2028	+ve	100	-ve	0	-ve	+ve	
29.	PC-2029	+ve	100	+ve	100	2.234	-ve	
30.	PC-2030	+ve	100	+ve	100	2.243	+ve	
31.	PC-2031	+ve	100	+ve	100	2.335	+ve	
32.	PC-2032	-ve	0	-ve	0	-ve	-ve	
33.	PC-2033	+ve	100	+ve	100	2.520	+ve	
34.	PC-2034	-ve	0	-ve	0	-ve	-ve	
35.	PC-2035	-ve	0	-ve	0	-ve	-ve	
36.	PC-2036	-ve	0	-ve	0	-ve	-ve	
37.	PC-2037	-ve	0	-ve	0	-ve	-ve	
38.	PC-2038	-ve	0	-ve	0	-ve	-ve	
39.	PC-2039	-ve	0	-ve	0	-ve	-ve	





40.	PC-2040	-ve	0	+ve	0	-ve	-ve	
41.	PC-2041	-ve	0	-ve	0	-ve	-ve	
42.	PC-2042	+ve	100	+ve	100	2.332	+ve	
43.	PC-2043	-ve	0	-ve	0	-ve	-ve	
44.	PC-2044	+ve	100	+ve	100	2.232	+ve	
45.	PC-2045	+ve	100	+ve	100	2.253	+ve	
46.	PC-2046	-ve	0	-ve	0	-ve	-ve	
47.	PC-2047	-ve	0	-ve	0	-ve	-ve	
48.	PC-2048	-ve	0	-ve	0	-ve	-ve	
49.	PC-2049	-ve	0	-ve	0	-ve	-ve	
50.	PC-2050	-ve	0	-ve	0	-ve	-ve	
51.	PC-2051	+ve	100	+ve	100	2.262	+ve	
52.	PC-2052	-ve	0	-ve	0	-ve	-ve	
53.	PC-2053	+ve	100	+ve	100	3.335	+ve	
54.	PC-2054	+ve	100	+ve	100	2.432	-ve	
55.	PC-2055	-ve	0	-ve	0	-ve	-ve	
56.	PC-2056	-ve	0	-ve	0	-ve	-ve	
57.	PC-2057	-ve	0	-ve	0	-ve	-ve	
58.	PC-2058	+ve	100	+ve	100	2.325	+ve	
59.	PC-2059	+ve	100	+ve	100	2.204	+ve	
60.	PC-2060	-ve	0	-ve	0	-ve	-ve	
61.	PC-2061	-ve	0	-ve	0	-ve	-ve	
62.	PC-2062	+ve	100	+ve	100	2.332	+ve	
63.	PC-2063	+ve	100	+ve	100	2.223	+ve	
64.	PC-2064	-ve	0	-ve	0	-ve	-ve	
65.	PC-2065	-ve	0	-ve	0	-ve	-ve	
66.	PC-2066	-ve	0	-ve	0	-ve	-ve	
67.	PC-2067	+ve	100	+ve	100	3.268	+ve	
68.	PC-2068	-ve	0	-ve	0	-ve	-ve	
69.	PC-2069	-ve	0	-ve	0	-ve	-ve	
70.	PC-2070	-ve	0	-ve	0	-ve	-ve	
71.	PC-2071	+ve	100	+ve	100	2.310	+ve	
72.	PC-2072	-ve	0	-ve	0	-ve	-ve	
73.	PC-2073	-ve	0	-ve	0	-ve	-ve	
74.	PC-2074	-ve	0	-ve	0	-ve	-ve	
75.	PC-2075	-ve	0	-ve	0	-ve	-ve	
76.	PC-2076	-ve	0	-ve	0	-ve	-ve	
77.	PC-2077	-ve	0	-ve	0	-ve	-ve	
78.	PC-2078	+ve	100	+ve	100	3.302	+ve	
79.	PC-2079	+ve	100	+ve	100	2.432	+ve	
80.	PC-2080	-ve	0	-ve	0	-ve	-ve	
81.	PC-2081	+ve	100	+ve	100	2.297	+ve	
82.	PC-2082	-ve	0	-ve	0	-ve	-ve	
83.	PC-2083	+ve	100	+ve	100	2.412	+ve	
84.	PC-2084	+ve	100	+ve	100	2.450	+ve	
85.	PC-2085	+ve	100	+ve	100	2.345	+ve	
86.	PC-2086	+ve	100	+ve	100	2.390	+ve	

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87.	PC-2087	-ve	0	-ve	0	-ve	-ve	
88.	PC-2088	-ve	0	-ve	0	-ve	-ve	
89.	PC-2089	+ve	100	+ve	100	2.316	+ve	
90.	PC-2090	+ve	100	+ve	100	2.221	+ve	
91.	PC-2091	+ve	100	+ve	100	2.543	+ve	
92.	PC-2092	-ve	0	-ve	0	-ve	-ve	
93.	PC-2093	-ve	0	-ve	0	-ve	-ve	
94.	PC-2094	-ve	0	-ve	0	-ve	-ve	
95.	PC-2095	+ve	100	+ve	100	2.532	+ve	
96.	PC-2096	+ve	100	+ve	100	2.352	+ve	
97.	PC-2097	-ve	0	-ve	0	-ve	-ve	
98.	PC-2098	+ve	100	+ve	100	2.242	+ve	
99.	PC-2099	+ve	100	+ve	100	2.332	+ve	
100.	PC-20100	-ve	0	-ve	0	-ve	-ve	
101.	PC-20101	-ve	0	-ve	0	-ve	-ve	
102.	PC-20102	-ve	0	-ve	0	-ve	-ve	
103.	PC-20103	+ve	100	+ve	100	2.331	+ve	
104.	PC-20104	-ve	0	-ve	0	-ve	-ve	
105.	PC-20105	-ve	0	-ve	0	-ve	-ve	
106.	PC-20106	+ve	100	+ve	100	2.222	+ve	
107.	PC-20107	+ve	100	+ve	100	2.325	+ve	
108.	PC-20108	+ve	100	+ve	100	2.402	+ve	
109.	PC-20109	+ve	100	+ve	100	2.252	+ve	
110.	PC-20110	+ve	100	+ve	100	2.275	+ve	


Done by


Checked by


Verified by



March 16, 2021

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Subject: 2020 NCVT Cotton Seed Samples for Biochemical Testing

Please refer to PCCC letter No. F.PCCC/DR(575) BCT-2020/469 dated 15 June, 2020 regarding biochemical testing of 110 cotton entries (PC-2001 to PC-2110). Please find attached cotton testing report of these cotton samples. We performed immuno-strip test for trait purity (%) and then event specific PCR of these 110 cotton lines.

Immuno-strip Test: To detect Cry1Ac, Cry2Ab and EPSPS proteins in the cotton samples, Immuno Strip test of each cotton line was conducted using QuickStix Combo Kit for Bollgard II / Roundup Ready (Cat No. AS046LS, Envirologix, USA).

PCR based Test: Cotton samples were also analyzed through PCR. Total genomic DNA was isolated from the subject samples of cotton using CTAB method. PCR was run and amplified products were resolved on agarose gel in the electrophoresis apparatus and visualized under UV light. Event specific PCR was performed using specific primers for cotton (Sad1, as an internal control), MON531 (Bollgard Bt cotton event of Monsanto containing Cry1Ac), Mon15985 (Monsanto BG-II Bt cotton event containing Cry1ac and Cry2ab) and Mon1445 (Roundup Ready cotton event of Monsanto containing CP4-EPSPS).

It is a matter of concern that number of testing samples is very large and it is not feasible to conduct these tests without proper funds. It is, therefore, desirable to devise a mechanism at PCCC level for arrangement of required funds or supply of testing materials to the testing labs. It will help to meet the expenditures done for these testing and also ensure the provision of such testing services in future.

Dr. Shahid Mansoor, SI
Director, NIBGE



2020 NCVT – BCT Test Results from NIBGE

2020 NCVT Cotton Seed Samples for Biochemical Testing were received from PCCC

Testing Results for Cry1Ac Gene (MON-531 Event) Bollgard-I Technology, Cry1ac + Cry2Ab Gene (MON-15985 Event) Bollgard-II Technology, CP4-EPSPS Gene (MON-1445 Event) RR Technology

Sr.	Codes	Cry1Ac (MON 531 or BG)			Cry2A (MON 15985 or BGII)			CP4-EPSPS (MON 1445 or RR)		
		Trait Confirmation (+/-) PCR	Trait Purity (%)	Quantification ELISA (µg/g)	Trait Confirmation (+/-) PCR	Trait Purity (%)	Quantification ELISA (µg/g)	Trait Confirmation (+/-) PCR	Trait Purity (%)	Quantification ELISA (µg/g)
1	PC-2001	-	0	NT	-	0	NT	-	0	NT
2	PC-2002	+	100	NT	-	0	NT	-	0	NT
3	PC-2003	+	20	NT	-	0	NT	-	0	NT
4	PC-2004	-	0	NT	-	0	NT	-	0	NT
5	PC-2005	-	0	NT	-	0	NT	-	0	NT
6	PC-2006	+	100	NT	-	0	NT	-	0	NT
7	PC-2007	-	0	NT	-	0	NT	-	0	NT
8	PC-2008	-	0	NT	-	0	NT	-	0	NT
9	PC-2009	+	100	NT	-	0	NT	-	0	NT
10	PC-2010	+	100	NT	-	0	NT	-	0	NT
11	PC-2011	+	100	NT	-	0	NT	+	100	NT
12	PC-2012	+	100	NT	-	0	NT	-	0	NT
13	PC-2013	+	100	NT	-	0	NT	-	0	NT
14	PC-2014	+	100	NT	-	0	NT	-	0	NT
15	PC-2015	+	100	NT	-	0	NT	-	0	NT
16	PC-2016	+	100	NT	-	0	NT	-	0	NT
17	PC-2017	+	100	NT	-	0	NT	-	0	NT
18	PC-2018	+	100	NT	-	0	NT	+	100	NT
19	PC-2019	+	100	NT	-	0	NT	-	0	NT
20	PC-2020	+	100	NT	-	0	NT	-	0	NT
21	PC-2021	+	20	NT	-	0	NT	-	0	NT
22	PC-2022	+	100	NT	-	0	NT	-	0	NT
23	PC-2023	+	100	NT	-	0	NT	-	0	NT
24	PC-2024	-	0	NT	-	0	NT	-	0	NT
25	PC-2025	+	100	NT	-	0	NT	-	0	NT



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26	PC-2026	+	100	NT	-	0	NT	-	0	NT
27	PC-2027	+	100	NT	-	0	NT	-	0	NT
28	PC-2028	+	100	NT	-	0	NT	-	0	NT
29	PC-2029	+	100	NT	-	0	NT	-	0	NT
30	PC-2030	+	100	NT	-	0	NT	-	0	NT
31	PC-2031	+	100	NT	+	100	NT	+	100	NT
32	PC-2032	+	100	NT	-	0	NT	-	0	NT
33	PC-2033	+	100	NT	-	0	NT	+	100	NT
34	PC-2034	+	100	NT	-	0	NT	-	0	NT
35	PC-2035	+	100	NT	-	0	NT	-	0	NT
36	PC-2036	+	100	NT	-	0	NT	-	0	NT
37	PC-2037	+	100	NT	-	0	NT	-	0	NT
38	PC-2038	+	100	NT	-	0	NT	-	0	NT
39	PC-2039	+	100	NT	-	0	NT	-	0	NT
40	PC-2040	+	100	NT	-	0	NT	-	0	NT
41	PC-2041	+	100	NT	-	0	NT	-	0	NT
42	PC-2042	+	100	NT	-	0	NT	-	100	NT
43	PC-2043	+	90	NT	-	0	NT	-	0	NT
44	PC-2044	+	100	NT	-	0	NT	+	100	NT
45	PC-2045	+	100	NT	-	0	NT	-	0	NT
46	PC-2046	+	100	NT	-	0	NT	-	0	NT
47	PC-2047	+	100	NT	-	0	NT	-	0	NT
48	PC-2048	+	100	NT	-	0	NT	-	0	NT
49	PC-2049	+	100	NT	-	0	NT	-	0	NT
50	PC-2050	+	100	NT	-	0	NT	-	0	NT
51	PC-2051	+	70	NT	-	0	NT	+	70	NT
52	PC-2052	+	100	NT	-	0	NT	-	0	NT
53	PC-2053	+	100	NT	-	0	NT	+	100	NT
54	PC-2054	+	100	NT	+	90	NT	+	100	NT
55	PC-2055	+	100	NT	-	0	NT	-	0	NT
56	PC-2056	+	100	NT	-	0	NT	-	0	NT
57	PC-2057	+	100	NT	-	0	NT	-	0	NT
58	PC-2058	+	100	NT	-	0	NT	+	100	NT
59	PC-2059	+	100	NT	-	0	NT	-	0	NT
60	PC-2060	+	100	NT	-	0	NT	-	0	NT
61	PC-2061	+	100	NT	-	0	NT	-	0	NT
62	PC-2062	+	90	NT	-	0	NT	+	90	NT
63	PC-2063	+	100	NT	-	0	NT	+	100	NT



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64	PC-2064	+	100	NT	-	0	NT	-	0	NT
65	PC-2065	+	100	NT	-	0	NT	-	0	NT
66	PC-2066	+	100	NT	-	0	NT	-	0	NT
67	PC-2067	+	100	NT	-	0	NT	-	0	NT
68	PC-2068	+	100	NT	-	0	NT	-	0	NT
69	PC-2069	+	100	NT	-	0	NT	-	0	NT
70	PC-2070	+	100	NT	-	0	NT	-	0	NT
71	PC-2071	+	100	NT	-	0	NT	+	100	NT
72	PC-2072	+	100	NT	-	0	NT	-	0	NT
73	PC-2073	+	100	NT	-	0	NT	-	0	NT
74	PC-2074	+	100	NT	-	0	NT	-	0	NT
75	PC-2075	+	100	NT	-	0	NT	+	100	NT
76	PC-2076	+	100	NT	-	0	NT	-	0	NT
77	PC-2077	+	70	NT	-	0	NT	-	0	NT
78	PC-2078	+	100	NT	-	0	NT	-	0	NT
79	PC-2079	+	100	NT	-	0	NT	-	0	NT
80	PC-2080	-	0	NT	-	0	NT	-	0	NT
81	PC-2081	+	100	NT	-	0	NT	+	100	NT
82	PC-2082	+	100	NT	-	0	NT	-	0	NT
83	PC-2083	+	100	NT	-	0	NT	-	0	NT
84	PC-2084	+	100	NT	-	0	NT	-	0	NT
85	PC-2085	+	100	NT	-	0	NT	-	0	NT
86	PC-2086	+	100	NT	-	0	NT	+	100	NT
87	PC-2087	-	0	NT	-	0	NT	-	0	NT
88	PC-2088	+	20	NT	-	0	NT	-	0	NT
89	PC-2089	+	100	NT	-	0	NT	-	0	NT
90	PC-2090	+	100	NT	-	0	NT	-	0	NT
91	PC-2091	+	100	NT	-	0	NT	-	0	NT
92	PC-2092	-	0	NT	-	0	NT	-	0	NT
93	PC-2093	+	100	NT	-	0	NT	-	0	NT
94	PC-2094	-	0	NT	-	0	NT	-	0	NT
95	PC-2095	-	0	NT	-	0	NT	-	0	NT
96	PC-2096	-	0	NT	-	0	NT	-	0	NT
97	PC-2097	+	100	NT	-	0	NT	-	0	NT
98	PC-2098	-	0	NT	-	0	NT	-	0	NT
99	PC-2099	+	50	NT	-	0	NT	-	0	NT
100	PC-2100	+	100	NT	-	0	NT	-	0	NT
101	PC-2101	+	100	NT	-	0	NT	-	0	NT



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102	PC-2102	+	100	NT	-	0	NT	-	0	NT
103	PC-2103	+	100	NT	-	0	NT	-	0	NT
104	PC-2104	+	100	NT	-	0	NT	-	0	NT
105	PC-2105	+	100	NT	-	0	NT	-	0	NT
106	PC-2106	+	100	NT	-	0	NT	+	100	NT
107	PC-2107	+	100	NT	-	0	NT	+	100	NT
108	PC-2108	+	100	NT	+	100	NT	+	100	NT
109	PC-2109	+	100	NT	-	0	NT	+	100	NT
110	PC-2110	+	100	NT	-	0	NT	+	100	NT

* Trait Confirmation (+/-) through event specific PCR; Trait Purity (%) through immuno-strip test;
NT= Not Tested (ELISA not performed due to unavailability of required kits)

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