pool (NHP) spiked with the Parvovirus B19 in-house standard to a genome titer of 5.0×10^3 IU/mL. The sensitivity requirement that the assay must detect positive samples within each individual test panel at a rate of 95 to 100% was met. In addition, the overall positive test rate for the 60 samples was 100% (Table 5).

The ability of the test to detect Parvovirus B19 at the targeted threshold value in the presence of feasible titers of the blood-borne DNA virus HBV was a test of assay robustness. As shown in Table 6, the acceptance criterion of a 95 to 100% test rate was met.

One robustness study in this validation involved testing a sample panel pool spiked with the Parvovirus B19 In-house Standard to a genome titer of 5.0×10^3 IU/mL extracted with 5% (w/v) CTAB that had been prepared from a different lot of Hexadecyltrimethylammonium Bromide (FisherChemical). The sensitivity requirement that the assay must detect positive samples within each test panel at a rate of 95 to 100% was met. The detection frequency in the panel was 100% (Table 7).

Another robustness component demonstrated the stability of the amplicon at the final 91°C HOLD step in the thermal-cycler. A sample panel pool was spiked with the Parvovirus B19 In-house Standard to a genome titer of 5.0×10^3 IU/mL. The extracts were amplified and the PCR amplification was held at 91°C for 60 minutes before aborting the amplification and proceeding with the detection. The sensitivity requirement that the assay must detect positive samples within each test panel at a rate of 95 to 100% was met (Table 8).

The potential of the assay to avoid cross-contamination events was addressed by extracting, amplifying, and detecting a panel of alternating negative samples (NHP) with 12 Parvovirus B19 samples at a titer of 5.0×10^5 IU/mL. Robustness of assay performance was assessed by the ability of the test to accurately detect negative (no positives) and B19 positive samples (100% positive) arranged in a checkerboard pattern (Figure 2). The acceptance criteria for this part of the validation were met as shown in Table 2.

Table 2 - Results of the Tests in the Study to Validate the Process for the Detection of Parvovirus B19 DNA in Donor Plasma Mini-Pools using Polymerase Chain Reaction Methodology, Version 2

Validation Level of Analyte Strength (Sample Type)	Type of Validation Testing	Acceptance Criteria	Results	Outcome
Negative diluent controls: donor plasma mini-pools	Specificity	No positives	0% positive*	VALID
Negative diluent controls: donor plasma mini-pools spiked with HBV Eurohep standard *adw* A at 5.0×10^5 EU/mL	Specificity	No positives	0% positive	VALID
In-house Test Panel: NHP spiked with Parvovirus B19 at 5.0×10^3 IU/mL	Sensitivity, robustness, repeatability and intermediate precision	95 to 100% positive	Op 1 100% Op 2 100% Op 3 100% Total 100%	VALID
In-house Test Panel: NHP spiked with Parvovirus B19 at 5.0×10^3 IU/mL and HBV Eurohep Standard *adw* A at 5×10^5 EU/mL	Robustness	95 to 100% positive	95% positive	VALID
In-house Test Panel: NHP spiked with Parvovirus B19 at 5.0×10^3 IU/mL - Samples extracted with 5% (w/v) CTAB prepared from a different lot of Hexadecyltrimethylammonium Bromide (FisherChemical)	Robustness	95 to 100% positive	100% positive	VALID
In-house Test Panel: NHP spiked with Parvovirus B19 at 5.0×10^3 IU/mL - Amplification HOLD for 60 minutes at 91°C prior to aborting the thermal cycling program	Robustness	95 to 100% positive	95% positive	VALID
Negative diluent control: NHP	Robustness (cross-contamination)	No positives	0% positive	VALID
In-house test panel: NHP spiked with Parvovirus B19 at 5.0×10^5 IU/mL	Robustness (cross-contamination)	100% positive	100% positive	VALID

* Panel member NAT-09910A was positive during the validation but was determined to have a low titer of Parvovirus B19. NAT-09910A was removed from the specificity panel as discussed above.

10. Conclusions

These studies verified that the Parvovirus B19 Plasma Donation Test, Version 2, using polymerase chain reaction methodology has the ability to consistently achieve a targeted cut-off value of 5.0×10^3 IU/mL in donor plasma pools with a positive test rate at or above 95%. This method includes a 1 mL CTAB/GITC nucleic acid sample preparation procedure coupled with target amplification using the Parvo B19 Probe/Primer set and target detection using the Digene *SHARP Signal* System Assay for PCR Products. Inclusion of Parvo B19 Internal Control DNA during sample preparation provides a control for nucleic acid recovery, sample transfer, amplification, and detection. The Parvovirus B19 Plasma Donation Test, Version 2, is suitable for testing donor plasma mini-pools, representing 96 to 480 plasma donations, for the presence of Parvovirus B19 DNA. This method is also applicable to the resolution and confirmatory phases of testing for Parvovirus B19-elevated pools. Implementation of the Parvovirus B19 Plasma Donation Test, Version 2, in addition to the Parvo B19 Test for Plasma Manufacturing Pools, will allow Talecris Biotherapeutics, Inc. to meet the PPTA Voluntary Standard for Parvovirus B19.

Table 3 - Absorbance values (405 nm) and Detection Frequency for the Test Panel for the Confirmation of Test Specificity

TEST PANEL: Parvovirus B19 - Negative Donor Plasma Mini-Pools			
Sample	**Operator**	**A_{405} Parvovirus B19**	**A_{405} Internal Control**
B19 NHP 082102	Operator 1	0.137	1.396
B19 IHP 082102	Operator 1	0.465	1.806
NAT-099A1	Operator 1	0.137	1.805
NAT-099A2	Operator 1	0.189	1.789
NAT-099A3	Operator 1	0.138	1.823
NAT-099A4	Operator 1	0.137	1.739
NAT-099A5	Operator 1	0.138	1.756
NAT-099A6	Operator 1	0.140	1.676
NAT-099A7	Operator 1	0.138	1.344
NAT-099A8	Operator 1	0.136	1.719
NAT-099A9	Operator 1	0.137	1.847
NAT-099A10	Operator 1	0.273	1.817
NAT-099A11	Operator 1	0.137	1.815
NAT-099A12	Operator 1	0.138	1.801
NAT-099A13	Operator 1	0.138	1.770
NAT-099A14	Operator 1	0.140	1.709
NAT-099A15	Operator 1	0.134	1.399
NAT-099A16	Operator 1	0.134	1.657
NAT-099A17	Operator 1	0.135	1.807
NAT-099A18	Operator 1	0.137	1.721
NAT-099A19	Operator 1	0.137	1.755
NAT-099A20	Operator 1	0.136	1.769
NAT-099A21	Operator 1	0.137	1.811
NAT-099A22	Operator 1	0.137	1.704
NAT-099A23	Operator 1	0.135	1.547
NAT-099A24	Operator 1	0.136	1.709
NAT-099A25	Operator 1	0.137	1.738
NAT-099A26	Operator 1	0.136	1.813
NAT-099A27	Operator 1	0.150	1.800
NAT-099A28	Operator 1	0.138	1.779
NAT-099A29	Operator 1	0.137	1.778
NAT-099A30	Operator 1	0.172	1.664
NAT-099A31	Operator 1	0.137	1.603
NAT-099A32	Operator 1	0.161	1.625
NAT-099A33	Operator 1	0.137	1.622

Table 3 - Continued

Sample	Operator	A_{405} Parvovirus B19	A_{405} Internal Control
B19 NHP 082102	Operator 2	0.140	0.538
B19 IHP 082102	Operator 2	0.329	0.883
NAT-099A34	Operator 2	0.138	1.119
NAT-099A35	Operator 2	0.139	1.326
NAT-099A36	Operator 2	0.139	1.311
NAT-099A37	Operator 2	0.139	1.278
NAT-099A38	Operator 2	0.158	1.012
NAT-099A39	Operator 2	0.139	0.603
NAT-099A40	Operator 2	0.140	0.968
NAT-099A41	Operator 2	0.138	1.265
NAT-099A42	Operator 2	0.139	1.331
NAT-099A43	Operator 2	0.139	1.429
NAT-099A44	Operator 2	0.138	1.333
NAT-099A45	Operator 2	0.138	1.357
NAT-099A46	Operator 2	0.138	1.293
NAT-099A47	Operator 2	0.139	1.229
NAT-099A48	Operator 2	0.139	0.741
NAT-099A49	Operator 2	0.140	1.176
NAT-099A50	Operator 2	0.138	1.273
NAT-099A51	Operator 2	0.138	1.445
NAT-099A52	Operator 2	0.151	1.447
NAT-099A53	Operator 2	0.214	1.367
NAT-099A54	Operator 2	0.154	1.350
NAT-099A55	Operator 2	0.141	1.252
NAT-099A56	Operator 2	0.139	0.934
NAT-099A57	Operator 2	0.138	1.202
NAT-099A58	Operator 2	0.139	1.299
NAT-099A59	Operator 2	0.138	1.383
NAT-099A60	Operator 2	0.138	1.255
NAT-099A61	Operator 2	0.138	1.393
NAT-099A62	Operator 2	0.142	1.270
NAT-099A63	Operator 2	0.145	0.755
NAT-099A64	Operator 2	0.139	0.817
NAT-099A65	Operator 2	0.138	1.070
NAT-099A66	Operator 2	0.138	1.153

Table 3 - Continued

Sample	Operator	A_{405} Parvovirus B19	A_{405} Internal Control
B19 NHP 082102	Operator 3	0.133	1.250
B19 IHP 082102	Operator 3	1.619	1.431
NAT-099A67	Operator 3	0.132	1.528
NAT-099A68	Operator 3	0.133	1.524
NAT-099A69	Operator 3	0.132	1.410
NAT-099A70	Operator 3	0.135	1.362
NAT-099A71	Operator 3	0.135	1.312
NAT-099A72	Operator 3	0.133	1.183
NAT-099A73	Operator 3	0.133	1.228
NAT-099A74	Operator 3	0.133	1.475
NAT-099A75	Operator 3	0.132	1.422
NAT-099A76	Operator 3	0.133	1.401
NAT-099A77	Operator 3	0.134	1.447
NAT-099A78	Operator 3	0.134	1.431
NAT-099A79	Operator 3	0.133	1.460
NAT-099A80	Operator 3	0.133	1.324
NAT-099A81	Operator 3	0.138	1.285
NAT-099A82	Operator 3	0.133	1.470
NAT-099A83	Operator 3	0.131	1.435
NAT-099A84	Operator 3	0.133	1.384
NAT-099A85	Operator 3	0.131	1.335
NAT-099A86	Operator 3	0.133	1.348
NAT-099A87	Operator 3	0.134	1.377
NAT-099A88	Operator 3	0.132	1.429
NAT-099A89	Operator 3	0.132	1.275
NAT-099A90	Operator 3	0.133	1.435
NAT-099A91	Operator 3	0.154	1.408
NAT-099A92	Operator 3	0.134	1.385
NAT-099A93	Operator 3	0.140	1.369
NAT-099A94	Operator 3	0.133	1.456
NAT-099A95	Operator 3	0.132	1.304
NAT-099A96	Operator 3	0.134	1.439
NAT-099A97	Operator 3	0.134	1.396
NAT-099A98	Operator 3	0.132	1.167
NAT-099A99	Operator 3	0.134	0.962
NAT-099A100	Operator 3	0.134	1.442
Positive Rate		1/100	100/100
Detection Frequency		1%	100%

Table 4 - Absorbance Values (405 nM) and Detection Frequency for the Test Panel for the Confirmation of Test Specificity in Donor Plasma Mini-Pools in the Presence of High-Titer HBV

TEST PANEL: Parvovirus B19 - Negative Initial Combined Samples Spiked with HBV to 5×10^5 EU/mL			
Sample	**Operator**	**A_{405} Parvovirus B19**	**A_{405} Internal Control**
B19 NHP 082102	Operator 3	0.132	1.246
B19 IHP 082102	Operator 3	1.401	1.270
NAT-099C1	Operator 3	0.132	1.373
NAT-099C2	Operator 3	0.133	1.322
NAT-099C3	Operator 3	0.132	1.285
NAT-099C4	Operator 3	0.134	1.227
NAT-099C5	Operator 3	0.134	1.314
NAT-099C6	Operator 3	0.136	1.129
NAT-099C7	Operator 3	0.133	1.259
NAT-099C8	Operator 3	0.131	1.428
NAT-099C9	Operator 3	0.131	1.318
NAT-099C10	Operator 3	0.132	1.356
NAT-099C11	Operator 3	0.132	1.250
NAT-099C12	Operator 3	0.131	1.312
NAT-099C13	Operator 3	0.133	1.219
NAT-099C14	Operator 3	0.132	1.217
NAT-099C15	Operator 3	0.133	1.362
NAT-099C16	Operator 3	0.131	1.486
NAT-099C17	Operator 3	0.131	1.253
NAT-099C18	Operator 3	0.131	1.286
NAT-099C19	Operator 3	0.133	1.408
NAT-099C20	Operator 3	0.132	1.397
Positive Rate		0/20	20/20
Detection Frequency		0%	100%

Table 5 - Absorbance Values (405 nM) and Detection Frequency for the Test Panel for the Confirmation of Test Sensitivity, Robustness, Repeatability and Intermediate Precision a Multiple-Source Plasma Pool Spiked with Parvovirus B19 to 5.0 x 10^3 IU/mL

TEST PANEL: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0 x 10^3 IU/mL						
Sample	**Operator 1**		**Operator 2**		**Operator 3**	
	A_{405} Parvovirus B19	**A_{405} Internal Control**	**A_{405} Parvovirus B19**	**A_{405} Internal Control**	**A_{405} Parvovirus B19**	**A_{405} Internal Control**
B19 NHP	0.135	1.695	0.148	0.649	0.141	1.346
B19 IHP 082102	1.096	1.741	0.488	0.981	0.760	1.392
NAT-099H1	1.181	1.816	0.891	1.227	0.820	1.244
NAT-099H2	1.253	1.760	0.982	1.236	0.759	1.157
NAT-099H3	1.360	1.746	0.850	1.277	0.872	1.156
NAT-099H4	1.284	1.806	0.923	1.294	0.719	1.395
NAT-099H5	1.139	1.771	0.780	1.142	1.815	1.335
NAT-099H6	0.971	1.523	0.511	0.852	0.961	0.995
NAT-099H7	0.988	1.634	0.458	0.609	1.547	1.172
NAT-099H8	1.178	1.684	0.917	1.088	0.905	1.358
NAT-099H9	1.115	1.810	1.064	1.461	0.691	1.245
NAT-099H10	1.267	1.759	1.038	1.783	0.782	1.197
NAT-099H11	1.323	1.787	1.123	1.695	1.442	0.964
NAT-099H12	1.214	1.728	1.279	1.744	1.421	0.948
NAT-099H13	1.210	1.748	1.141	1.516	1.433	1.081
NAT-099H14	1.102	1.744	0.497	1.130	0.645	0.892
NAT-099H15	0.899	1.787	0.403	0.832	1.291	0.850
NAT-099H16	1.110	1.782	0.846	1.320	0.790	1.200
NAT-099H17	1.306	1.735	1.022	1.836	1.005	1.206
NAT-099H18	1.186	1.822	1.075	1.872	0.755	1.205
NAT-099H19	1.079	1.782	1.049	1.795	0.867	1.230
NAT-099H20	0.978	1.787	0.863	1.869	0.833	1.180
Positive Rate	20 /20	20 /20	20 /20	20 /20	20 /20	20 /20
Total Positive Rate	60/60	60/60				
Detection Frequency	100%	100%	100%	100%	100%	100%
Total Detection Frequency	100%	100%				

Table 6 - Absorbance Values (405 Nm) and Detection Frequency for the Test Panel (Parvovirus B19 at 5.0 X 10^3 IU/mL) for the Confirmation of Test Robustness in a Multiple-Source Plasma Pool in the Presence of High-Titer HBV

TEST PANEL: NHP (NAT-094) spiked with Parvovirus B19 to 5.0 x 10^3 IU/mL and HBV to 5 x 10^5 EU/mL			
Sample	**Operator**	**A_{405} Parvovirus B19**	**A_{405} Internal Control**
B19 NHP 082102	Operator 2	0.143	0.955
B19 IHP 082102	Operator 2	0.441	1.394
NAT-09911	Operator 2	0.863	1.606
NAT-09912	Operator 2	0.716	1.663
NAT-09913	Operator 2	0.719	1.578
NAT-09914	Operator 2	0.685	1.564
NAT-09915	Operator 2	0.426	1.260
NAT-09916	Operator 2	0.243	1.068
NAT-09917	Operator 2	0.343	0.841
NAT-09918	Operator 2	0.460	1.536
NAT-09919	Operator 2	0.671	1.499
NAT-099110	Operator 2	0.571	1.589
NAT-099111	Operator 2	0.612	1.605
NAT-099112	Operator 2	0.489	1.602
NAT-099113	Operator 2	0.394	1.386
NAT-099114	Operator 2	0.176	0.752
NAT-099115	Operator 2	0.318	0.901
NAT-099116	Operator 2	0.439	1.526
NAT-099117	Operator 2	0.532	1.563
NAT-099118	Operator 2	0.478	1.728
NAT-099119	Operator 2	0.414	1.516
NAT-099120	Operator 2	0.384	1.573
Positive Rate		19/20	20/20
Detection Frequency		95%	100%

Table 7 - Absorbance Values (405 nM) and Detection Frequency for the Test Panel (Parvovirus B19 at 5.0 X 10^3 IU/mL) for the Confirmation of Test Robustness for Samples Extracted With 5% (W/V) CTAB Prepared from a Different Lot of Hexadecyltrimethylammonium Bromide (FisherChemical)

TEST PANEL: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0 x 10^3 IU/mL			
Sample	**Operator**	**A_{405} Parvovirus B19**	**A_{405} Internal Control**
B19 NHP 082102	Operator 2	0.137	1.000
B19 IHP 082102	Operator 2	0.626	1.623
NAT-099H1	Operator 2	0.884	1.723
NAT-099H2	Operator 2	1.009	1.823
NAT-099H3	Operator 2	1.032	1.732
NAT-099H4	Operator 2	0.932	1.794
NAT-099H5	Operator 2	0.839	1.627
NAT-099H6	Operator 2	0.445	1.282
NAT-099H7	Operator 2	0.670	1.363
NAT-099H8	Operator 2	0.827	1.538
NAT-099H9	Operator 2	0.907	1.807
NAT-099H10	Operator 2	0.873	1.862
NAT-099H11	Operator 2	0.903	1.713
NAT-099H12	Operator 2	0.774	1.814
NAT-099H13	Operator 2	0.514	1.624
NAT-099H14	Operator 2	0.294	1.187
NAT-099H15	Operator 2	0.455	1.283
NAT-099H16	Operator 2	0.494	1.508
NAT-099H17	Operator 2	0.644	1.647
NAT-099H18	Operator 2	0.761	1.763
NAT-099H19	Operator 2	0.642	1.632
NAT-099H20	Operator 2	0.578	1.655
Positive Rate		20/20	20/20
Detection Frequency		100%	100%

Table 8 - Absorbance Values (405 nM) and Detection Frequency for the Test Panel (Parvovirus B19 at 5.0 X 10^3 IU/mL) for the Confirmation of Test Robustness with the Amplification HOLD for 60 Minutes at 91°C Prior to Aborting the Thermal Cycling Program

TEST PANEL: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0 x 10^5 IU/mL			
Sample	**Operator**	**A_{405} Parvovirus B19**	**A_{405} Internal Control**
B19 NHP 082102	Operator 1	0.140	1.138
B19 IHP 082102	Operator 1	0.531	1.529
NAT-099H1	Operator 1	0.740	1.460
NAT-099H2	Operator 1	0.920	1.622
NAT-099H3	Operator 1	0.962	1.607
NAT-099H4	Operator 1	0.825	1.413
NAT-099H5	Operator 1	0.719	1.508
NAT-099H6	Operator 1	0.884	1.413
NAT-099H7	Operator 1	0.285	0.930
NAT-099H8	Operator 1	0.734	1.255
NAT-099H9	Operator 1	0.806	1.346
NAT-099H10	Operator 1	1.130	1.378
NAT-099H11	Operator 1	1.117	1.495
NAT-099H12	Operator 1	1.030	1.482
NAT-099H13	Operator 1	1.081	1.431
NAT-099H14	Operator 1	0.970	1.308
NAT-099H15	Operator 1	0.211	0.866
NAT-099H16	Operator 1	0.656	1.340
NAT-099H17	Operator 1	1.128	1.337
NAT-099H18	Operator 1	1.061	1.293
NAT-099H19	Operator 1	0.821	1.348
NAT-099H20	Operator 1	0.799	1.201
Positive Rate		19/20	20/20
Detection Frequency		95%	100%

Figure 2 - Sample detection array with test results for the cross-contamination study. As in Figure 1, sample identity and coordinates are defined according to their placement in the detection microwell plate. The hybridization targets are indicated in the column headings; "B19" indicates that the samples in these columns are detected with the Parvovirus B19-specific RNA probe, whereas "IC" indicates that the samples in these columns are detected with the Parvo B19 internal control-specific RNA probe. B19 and IC columns are paired to match target and internal control as follows: 1 and 4, 2 and 5, 3 and 6. For Parvovirus B19-specific reactions, a "+" indicates a positive detection and a "–" indicates a negative detection. Raw absorbance values (405 nM) for the colorimetric assay are shown in parentheses.

	1 (B19)	2 (B19)	3 (B19)	4 (IC)	5 (IC)	6 (IC)
A	NAT-099J1 – (0.141)	NAT-099K5 + (1.418)	NAT-099J9 – (0.131)	NAT-099J1 (1.407)	NAT-099K5 (1.214)	NAT-099J9 (1.185)
B	NAT-099K1 + (1.674)	NAT-099J5 – (0.130)	NAT-099K9 + (1.839)	NAT-099K1 (1.490)	NAT-099J5 (1.765)	NAT-099K9 (1.388)
C	NAT-099J2 – (0.141)	NAT-099K6 + (1.617)	NAT-099J10 – (0.131)	NAT-099J2 (1.833)	NAT-099K6 (1.352)	NAT-099J10 (1.654)
D	NAT-099K2 + (1.431)	NAT-099J6 – (0.158)	NAT-099K10 + (1.536)	NAT-099K2 (1.276)	NAT-099J6 (1.752)	NAT-099K10 (1.353)
E	NAT-099J3 – (0.139)	NAT-099K7 + (1.428)	NAT-099J11 – (0.131)	NAT-099J3 (1.749)	NAT-099K7 (1.182)	NAT-099J11 (1.733)
F	NAT-099K3 + (1.493)	NAT-099J7 – (0.128)	NAT-099K11 + (1.506)	NAT-099K3 (1.231)	NAT-099J7 (1.597)	NAT-099K11 (1.283)
G	NAT-099J4 – (0.183)	NAT-099K8 + (1.528)	NAT-099J12 – (0.143)	NAT-099J4 (1.594)	NAT-099K8 (1.161)	NAT-099J12 (1.618)
H	NAT-099K4 + (1.678)	NAT-099J8 – (0.129)	NAT-099K12 + (1.587)	NAT-099K4 (1.162)	NAT-099J8 (1.484)	NAT-099K12 (1.176)

11. Appendix A

Preparation of controls for the validation of the method for the detection of Parvovirus B19 DNA in donor plasma pools using polymerase chain reaction methodology, version 2.

Form for Preparation of Controls for the Parvovirus B19 DNA Donor Plasma Screening Test, Version 2, NAT Development Lab
Bayer Corporation, Biological Products
Raleigh Test Laboratory

Document #: AF 682.002M

PREPARATION OF CONTROLS FOR THE PARVOVIRUS B19 DNA DONOR PLASMA SCREENING TEST, VERSION 2

Analyst: Anne Keen / Marissa Williams Date: 8/21/02

In-house Negative Control (NHP)

Lot Number: B19 NHP 082102

NAT DEV stock number for NHP: NAT- 094

Number of 1 mL aliquots prepared: 34

In-house Positive Control (IHP) at 5 x 10^3 IU/mL

Lot Number: B19 IHP 082102

NAT DEV stock number for Parvovirus B19 In-house Standard: NAT- 056

Concentration of Parvovirus B19 In-house Standard: 7.6×10^{11} IU/mL

NAT DEV stock number for NHP diluent: NAT- 094

Number of 1 mL aliquots prepared: 28

Volume of stock	Stock source & Concentration	Volume of diluent	Diluent source & Concentration	Volume of sample	Sample & Concentration
230µl	7.6×10^{11} IU/mL	120µl	NHP (NAT-094)	350µl	5.0×10^{11} IU/mL
20µl	5.0×10^{8} IU/mL	1.98 mL	NHP (NAT-094)	2.0 mL	5.0×10^{6} IU/mL
20µl	5.0×10^{6} IU/mL	1.98 mL	NHP (NAT-094)	2.0mL	5.0×10^{4} IU/mL
20µl	5.0×10^{4} IU/mL	1.98 mL	NHP (NAT-094)	2.0mL	5.0×10^{2} IU/mL
320µl	5.0×10^{5} IU/mL	31.68 mL	NHP (NAT-094)	32.0mL	5.0×10^{3} IU/mL

Comments:

12. Appendix B

Preparation of intermediates and test panels for the validation of the process for the detection of Parvovirus B19 DNA in donor plasma pools using polymerase chain reaction methodology, version 2

Table B.1. - Preparation of the Test Panel for the Confirmation of Test Specificity in Parvovirus B19 DNA-Negative Pools

Test Panel: Parvovirus B19 - Negative Donor Plasma Mini-Pools - Negative Diluent Panel					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099A1	N/A	N/A	1 mL	020723A8C	1
NAT-099A2	N/A	N/A	1 mL	020723A9C	1
NAT-099A3	N/A	N/A	1 mL	020723A7A	1
NAT-099A4	N/A	N/A	1 mL	020723A9B	1
NAT-099A5	N/A	N/A	1 mL	020723A6A	1
NAT-099A6	N/A	N/A	1 mL	020723D2A	1
NAT-099A7	N/A	N/A	1 mL	020722P3E	1
NAT-099A8	N/A	N/A	1 mL	020722P2E	1
NAT-099A9	N/A	N/A	1 mL	020723A4B	1
NAT-099A10	N/A	N/A	1 mL	020723A3E	1
NAT-099A11	N/A	N/A	1 mL	020722R1D	1
NAT-099A12	N/A	N/A	1 mL	020722R1C	1
NAT-099A13	N/A	N/A	1 mL	020723A8D	1
NAT-099A14	N/A	N/A	1 mL	020723A8A	1
NAT-099A15	N/A	N/A	1 mL	020723A7C	1
NAT-099A16	N/A	N/A	1 mL	020723A6B	1
NAT-099A17	N/A	N/A	1 mL	020722P3D	1
NAT-099A18	N/A	N/A	1 mL	020722P2B	1
NAT-099A19	N/A	N/A	1 mL	020723A4A	1
NAT-099A20	N/A	N/A	1 mL	020723A3D	1
NAT-099A21	N/A	N/A	1 mL	020722P5A	1
NAT-099A22	N/A	N/A	1 mL	020722R1B	1
NAT-099A23	N/A	N/A	1 mL	020723A8E	1
NAT-099A24	N/A	N/A	1 mL	020723A8B	1
NAT-099A25	N/A	N/A	1 mL	020723A7D	1
NAT-099A26	N/A	N/A	1 mL	020723A6C	1
NAT-099A27	N/A	N/A	1 mL	020722P3C	1
NAT-099A28	N/A	N/A	1 mL	020722P2A	1

Table B.1. - Continued

Test Panel: Parvovirus B19 - Negative Donor Plasma Mini-Pools - Negative Diluent Panel					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099A29	N/A	N/A	1 mL	020723A4D	1
NAT-099A30	N/A	N/A	1 mL	020723A3C	1
NAT-099A31	N/A	N/A	1 mL	020722P6A	1
NAT-099A32	N/A	N/A	1 mL	020723A6D	1
NAT-099A33	N/A	N/A	1 mL	020724P3A	1
NAT-099A34	N/A	N/A	1 mL	020723A7E	1
NAT-099A35	N/A	N/A	1 mL	020723A4C	1
NAT-099A36	N/A	N/A	1 mL	020722P2D	1
NAT-099A37	N/A	N/A	1 mL	020722P3B	1
NAT-099A38	N/A	N/A	1 mL	020723A2A	1
NAT-099A39	N/A	N/A	1 mL	020723A3B	1
NAT-099A40	N/A	N/A	1 mL	020723D2E	1
NAT-099A41	N/A	N/A	1 mL	020724P2A	1
NAT-099A42	N/A	N/A	1 mL	020723A5B	1
NAT-099A43	N/A	N/A	1 mL	020723A4E	1
NAT-099A44	N/A	N/A	1 mL	020722P2C	1
NAT-099A45	N/A	N/A	1 mL	020722P3A	1
NAT-099A46	N/A	N/A	1 mL	020723A1D	1
NAT-099A47	N/A	N/A	1 mL	020723A2C	1
NAT-099A48	N/A	N/A	1 mL	020723A3A	1
NAT-099A49	N/A	N/A	1 mL	020723D6A	1
NAT-099A50	N/A	N/A	1 mL	020723D9A	1
NAT-099A51	N/A	N/A	1 mL	020723A5C	1
NAT-099A52	N/A	N/A	1 mL	020723P1A	1
NAT-099A53	N/A	N/A	1 mL	020723D4A	1
NAT-099A54	N/A	N/A	1 mL	020723D3A	1
NAT-099A55	N/A	N/A	1 mL	020723A1C	1
NAT-099A56	N/A	N/A	1 mL	020723A2E	1
NAT-099A57	N/A	N/A	1 mL	020723P1D	1
NAT-099A58	N/A	N/A	1 mL	020723D6B	1
NAT-099A59	N/A	N/A	1 mL	020723D6C	1
NAT-099A60	N/A	N/A	1 mL	020723A5D	1
NAT-099A61	N/A	N/A	1 mL	020723D4B	1
NAT-099A62	N/A	N/A	1 mL	020723D3C	1
NAT-099A63	N/A	N/A	1 mL	020723A1B	1
NAT-099A64	N/A	N/A	1 mL	020723P1C	1

Table B.1. - Continued

Test Panel: Parvovirus B19 - Negative Donor Plasma Mini-Pools - Negative Diluent Panel					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099A65	N/A	N/A	1 mL	020725B5A	1
NAT-099A66	N/A	N/A	1 mL	020723D6D	1
NAT-099A67	N/A	N/A	1 mL	020723A5E	1
NAT-099A68	N/A	N/A	1 mL	020723D4C	1
NAT-099A69	N/A	N/A	1 mL	020723D3E	1
NAT-099A70	N/A	N/A	1 mL	020723D3D	1
NAT-099A71	N/A	N/A	1 mL	020723A1A	1
NAT-099A72	N/A	N/A	1 mL	020723P1B	1
NAT-099A73	N/A	N/A	1 mL	020723B3D	1
NAT-099A74	N/A	N/A	1 mL	020723D8A	1
NAT-099A75	N/A	N/A	1 mL	020723A9D	1
NAT-099A76	N/A	N/A	1 mL	020723A9E	1
NAT-099A77	N/A	N/A	1 mL	020723B3B	1
NAT-099A78	N/A	N/A	1 mL	020723B3A	1
NAT-099A79	N/A	N/A	1 mL	020724B9A	1
NAT-099A80	N/A	N/A	1 mL	020724B7D	1
NAT-099A81	N/A	N/A	1 mL	020724B5E	1
NAT-099A82	N/A	N/A	1 mL	020724B5C	1
NAT-099A83	N/A	N/A	1 mL	020724B4E	1
NAT-099A84	N/A	N/A	1 mL	020724B4C	1
NAT-099A85	N/A	N/A	1 mL	020724B7C	1
NAT-099A86	N/A	N/A	1 mL	020724B7B	1
NAT-099A87	N/A	N/A	1 mL	020724B7A	1
NAT-099A88	N/A	N/A	1 mL	020724B7E	1
NAT-099A89	N/A	N/A	1 mL	020724B6E	1
NAT-099A90	N/A	N/A	1 mL	020724B6B	1
NAT-099A91	N/A	N/A	1 mL	020724B6A	1
NAT-099A92	N/A	N/A	1 mL	020724D1A	1
NAT-099A93	N/A	N/A	1 mL	020724B5B	1
NAT-099A94	N/A	N/A	1 mL	020724B5A	1
NAT-099A95	N/A	N/A	1 mL	020724B4A	1
NAT-099A96	N/A	N/A	1 mL	020724B6D	1
NAT-099A97	N/A	N/A	1 mL	020724B6C	1
NAT-099A98	N/A	N/A	1 mL	020724B4D	1
NAT-099A99	N/A	N/A	1 mL	020724B4B	1
NAT-099A100	N/A	N/A	1 mL	020722R1A	1

Table B.2. - Preparation of the HBV Intermediate and Test Panel for the Confirmation of Test Specificity in Parvovirus B19 DNA-Negative Pools Spiked with HBV at 5 X 10^5 EU/mL

Test Panel: Parvovirus B19 - Negative Initial Combined Samples Spiked with HBV to 5 x 10^5 EU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099B HBV Intermediate at 5 x 10^7 EU/mL	18.5 µL	HBV Standard *adw*/Genotype A at 2.7 x 10^9 EU/mL	981.5 µL	NAT-094	1
NAT-099C1	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020723D2D	1
NAT-099C2	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020723A1E	1
NAT-099C3	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A2A	1
NAT-099C4	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A2B	1
NAT-099C5	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A2C	1
NAT-099C6	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A2D	1
NAT-099C7	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A2E	1
NAT-099C8	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A3A	1
NAT-099C9	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A3C	1
NAT-099C10	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A3D	1
NAT-099C11	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A3E	1
NAT-099C12	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A1B	1
NAT-099C13	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A1C	1
NAT-099C14	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A6A	1
NAT-099C15	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A5C	1

Table B.2. - Continued

Test Panel: Parvovirus B19 - Negative Initial Combined Samples Spiked with HBV to 5 x 10^5 EU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099C16	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A5D	1
NAT-099C17	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A5E	1
NAT-099C18	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A5B	1
NAT-099C19	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A5A	1
NAT-099C20	10 µL	HBV Intermediate at 5 x 10^7 EU/mL	990 µL	020802A4E	1

Table B.3. - Preparation of the Parvovirus B19 Intermediates and Test Panel for the Confirmation of Test Sensitivity, Robustness, Repeatability, and Intermediate Precision in a Parvovirus B19 DNA-Negative Multiple-Source Plasma Pool Spiked with Parvovirus B19 to 5.0×10^3 IU/mL

Test Panel: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0×10^3 IU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099D B19 Intermediate at 5.0×10^{11} IU/mL	230 µL	NAT-056 at 7.6×10^{11} IU/mL	120 µL	NAT-094	1/operator x 3
NAT-099E B19 Intermediate at 5.0×10^{9} IU/mL	20 µL	B19 Intermediate at 5.0×10^{11} IU/mL	1.98 mL	NAT-094	1/operator x 3
NAT-099F B19 Intermediate at 5.0×10^{7} IU/mL	20 µL	B19 Intermediate at 5.0×10^{9} IU/mL	1.98 mL	NAT-094	1/operator x 3
NAT-099G B19 Intermediate at 5.0×10^{5} IU/mL	20 µL	B19 Intermediate at 5.0×10^{7} IU/mL	1.98 mL	NAT-094	1/operator x 3
NAT-099H B19 panel at 5.0×10^{4} IU/mL	320 µL	B19 Intermediate at 5.0×10^{5} IU/mL	31.68 mL	NAT-094	1/operator x 3
NAT-099H1	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H2	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H3	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H4	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H5	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H6	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H7	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H8	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H9	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H10	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3
NAT-099H11	N/A	NAT-099H at 5.0×10^{3} IU/mL	N/A	N/A	1/operator x 3

Table B.3. - Continued

Test Panel: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0 x 10^3 IU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099H12	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1/operator x 3
NAT-099H13	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1/operator x 3
NAT-099H14	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1/operator x 3
NAT-099H15	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1/operator x 3
NAT-099H16	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1/operator x 3
NAT-099H17	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1/operator x 3
NAT-099H18	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1/operator x 3
NAT-099H19	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1/operator x 3
NAT-099H20	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1/operator x 3

Table B.4. - Preparation of the Parvovirus B19 Test Panel for the Confirmation of Test Robustness in a Parvovirus B19 DNA-Negative Multiple-Source Plasma Pool Spiked with Parvovirus B19 to 5.0×10^3 IU/mL - Samples Extracted with 5% (w/v) CTAB Prepared from a Different Lot of Hexadecyltrimethylammonium Bromide (FisherChemical).

Test Panel: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0×10^3 IU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099H B19 Panel at 5.0×10^3 IU/mL	550 µL	B19 Intermediate at 5.0×10^5 IU/mL	54.45 mL	NAT-094	1
NAT-099H1	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H2	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H3	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H4	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H5	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H6	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H7	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H8	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H9	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H10	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H11	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H12	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H13	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H14	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H15	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1
NAT-099H16	N/A	NAT-099H at 5.0×10^3 IU/mL	N/A	N/A	1

Table B.4. - Continued

Test Panel: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0 x 10^3 IU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099H17	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1
NAT-099H18	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1
NAT-099H19	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1
NAT-099H20	N/A	NAT-099H at 5.0 x 10^3 IU/mL	N/A	N/A	1

Table B.5. - Preparation of the Test Panel for the Confirmation of Test Robustness in a Parvovirus B19 DNA-Negative Multiple-Source Plasma Pool Spiked with Parvovirus B19 to 5.0×10^3 IU/mL and HBV to 5.0×10^5 EU/mL

Test Panel: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0×10^3 IU/mL and HBV to 5.0×10^5 EU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099H B19 Intermediate at 5.0×10^3 IU/mL	550 µL	B19 Intermediate at 5.0×10^5 IU/mL	54.45 mL	NAT-094	1
NAT-099I B19 at 5.0×10^3 IU/mL and HBV at 5×10^5 EU/mL	250 µL	HBV Intermediate at 5×10^7 EU/mL	24.75 mL	NAT-099H B19 Intermediate at 5.0×10^3 IU/mL	1
NAT-099I1	N/A	NAT-099I1 B19 at 5.0×10^3 IU/mL and HBV at 5×10^5 EU/mL	N/A	N/A	1
NAT-099I2	N/A	NAT-099I1 B19 at 5.0×10^3 IU/mL and HBV at 5×10^5 EU/mL	N/A	N/A	1
NAT-099I3	N/A	NAT-099I1 B19 at 5.0×10^3 IU/mL and HBV at 5×10^5 EU/mL	N/A	N/A	1
NAT-099I4	N/A	NAT-099I1 B19 at 5.0×10^3 IU/mL and HBV at 5×10^5 EU/mL	N/A	N/A	1
NAT-099I5	N/A	NAT-099I1 B19 at 5.0×10^3 IU/mL and HBV at 5×10^5 EU/mL	N/A	N/A	1
NAT-099I6	N/A	NAT-099I1 B19 at 5.0×10^3 IU/mL and HBV at 5×10^5 EU/mL	N/A	N/A	1
NAT-099I7	N/A	NAT-099I1 B19 at 5.0×10^3 IU/mL and HBV at 5×10^5 EU/mL	N/A	N/A	1

Table B.5. - Continued

Test Panel: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0 x 10^3 IU/mL and HBV to 5.0 x 10^5 EU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099I8	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I9	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I10	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I11	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I12	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I13	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I14	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I15	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I16	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I17	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I18	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1

Table B.5. - Continued

Test Panel: NHP (NAT-094) Spiked with Parvovirus B19 to 5.0 x 10^3 IU/mL and HBV to 5.0 x 10^5 EU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099I19	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1
NAT-099I20	N/A	NAT-099I1 B19 at 5.0 x 10^3 IU/mL and HBV at 5 x 10^5 EU/mL	N/A	N/A	1

Table B.6. - Preparation of the Parvovirus B19-Negative Sample Test Panel for the Confirmation of Test Robustness (Cross-Contamination)

Test Panel: Parvovirus B19 - Negative Sample Panel					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099J1	N/A	N/A	1 mL	NAT-094	1
NAT-099J2	N/A	N/A	1 mL	NAT-094	1
NAT-099J3	N/A	N/A	1 mL	NAT-094	1
NAT-099J4	N/A	N/A	1 mL	NAT-094	1
NAT-099J5	N/A	N/A	1 mL	NAT-094	1
NAT-099J6	N/A	N/A	1 mL	NAT-094	1
NAT-099J7	N/A	N/A	1 mL	NAT-094	1
NAT-099J8	N/A	N/A	1 mL	NAT-094	1
NAT-099J9	N/A	N/A	1 mL	NAT-094	1
NAT-099J10	N/A	N/A	1 mL	NAT-094	1
NAT-099J11	N/A	N/A	1 mL	NAT-094	1
NAT-099J12	N/A	N/A	1 mL	NAT-094	1

Table B.7. - Preparation of the Parvovirus B19-Positive Sample Test Panel for the Confirmation of Test Robustness (Cross-Contamination)

Test Panel: Parvovirus B19 - Positive Sample Panel spiked to 5.0 x 10^5 IU/mL					
Validation Panel Member	**Volume of Stock**	**Stock Source**	**Volume of Diluent**	**Diluent Source**	**Number of Samples**
NAT-099K B19 Panel at 5.0 x 10^5 IU/mL	160 µL	B19 Intermediate at 5.0 x 10^7 IU/mL	15.84 mL	NAT-094	1
NAT-099K1	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K2	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K3	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K4	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K5	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K6	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K7	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K8	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K9	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K10	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K11	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1
NAT-099K12	N/A	NAT-099K at 5.0 x 10^5 IU/mL	N/A	N/A	1

13. Appendix C

Results of the characterization study to determine the sensitivity of the method for the detection of Parvovirus B19 DNA in donor plasma pools, version 2, using polymerase chain reaction methodology.

13.1 Objective

The aim of this study was to determine the Parvovirus B19 genome titer corresponding to the 95 and 99% positive test rates of the method for the detection of Parvovirus B19 DNA in donor plasma pools, Version 2, using polymerase chain reaction methodology.

13.2 Methods

The Parvovirus B19 In-house Standard (NAT-056) was used to prepare a dilution panel.[6] The dilution panel was comprised of samples at the following titers: 1.0×10^4 IU/mL, 5.0×10^3 IU/mL, 3.2×10^3 IU/mL, 1.0×10^3 IU/mL, 3.2×10^2 IU/mL and 3.2×10^1 IU/mL. Diluent for the dilution panel was a multiple-source plasma pool (NAT-094) which was found non-reactive for HIV-1, HCV, HBV and Parvovirus B19 using PCR methodologies. Three operators tested four samples at each viral titer ($n_{total} = 12$) using the Parvovirus B19 Plasma Donation Test, Version 2.

13.3 Results

The results of the sensitivity study are shown in Table C.1. The 99% detection frequency for the test was predicted using the model for the binary response data, Probit. Based on the normal cumulative distribution function, the Probit analysis predicts that samples with a Parvovirus B19 titer of 1.7×10^3 IU/mL will be detected 99% of the time.

13.4 Conclusions

The 99% positive rate for the method for the detection for Parvovirus B19 DNA in donor plasma pools using polymerase chain reaction methodology, Version 2, is 1.7×10^3 IU/mL. This is approximately three times more sensitive than the targeted cut-off of 5.0×10^3 IU/mL.

Table C.1. - Study to Determine the Sensitivity of the Parvovirus B19 In-House Standard with the Parvovirus B19 Plasma Donation Test, Version 2

Parvovirus B19 Titer (IU/mL)	Operator 1 Number Positive	Operator 2 Number Positive	Operator 3 Number Positive	n Size	Total Number Positive	Average B19 A405
1.0 E + 04	4	1	4	9	9	0.986
5.0 E + 03	4	2	4	10	10	0.896
3.2 E + 03	4	4	4	12	12	0.806
1.0 E + 03	4	3	2	12	9	0.504
320	2	3	3	12	8	0.319
32	0	0	0	12	0	0.156
		Probit Analysis Detection frequency		95%	1367	
				99%	**1728**	

14. Appendix D

Positive result investigation for specificity panel member NAT-099A10

Table D.1. - Repeat Amplification and Detection of Specificity Panel Member NAT-099A10 using the Digene *SHARP Signal*™ System Assay for PCR Products

Sample	Operator	A_{405} Parvovirus B19	A_{405} Internal Control
B19 NHP 082102	Operator 1	0.150	1.386
B19 IHP 082102	Operator 1	0.406	1.525
NAT-099A10	Operator 1	0.150	1.514
NAT-099A10	Operator 1	0.167	1.654
NAT-099A10	Operator 1	0.149	1.564
NAT-099A10	Operator 1	0.154	1.617
Positive Rate		0/4	4/4
Detection Frequency		0%	100%

Table D.2. - Quantitation of Parvovirus B19 DNA in Specificity Panel Member NAT-099A10 using Fluorogenic PCR Technology

Sample	Operator	FAM C_T Values	Quantity (copies/mL)
NAT-099A10	Operator 1	38.8	3.6 E + 01
NAT-099A10	Operator 1	40.0	Below Detection Limit
NAT-099A10	Operator 1	40.0	Below Detection Limit
NAT-099A10	Operator 1	39.5	2.3 E + 01
Positive Rate		2/4	
Detection Frequency		50%	

15. Appendix E

15.1 Strategy for the Adjustment and Implementation of Controls

Detection Controls

Detection Controls including the Positive Assay Control (PAC), Parvo B19 IC PAC, and the Negative Assay Control (NAC) are used for troubleshooting and quality control functions. They have not been incorporated into the testing algorithm for donor sample mini-pools. Their implementation is discretionary; however, they should be implemented in a manner consistent with the manufacturer recommendation.

Assay Validation Controls

Test disposition and assessment of control performance are governed by the determination of the positive cut-off value. The positive cut-off value is set dynamically by determining the assay background signal and adding 0.100. The assay signal background is the A_{405} signal for the normal human plasma (NHP) negative control of the run - provided that the NHP signal is valid (i.e. <0.250). The stringency of assay validation controls may be increased by setting a static cut-off at some point above 0.250. There may be particular instances when a specific cut-off is desired to increase the performance requirements of a particular function of the assay. For example, performance requirements for internal controls may be increased by raising the acceptance cut-off above the range of the typical values for the positive cut-off value. Such an increase in the stringency of this performance requirement would likely result in an increase in the assay failure rate, leading to a higher rate for retesting of negative samples. Therefore, such adjustments must be statistically justified through the survey and analysis of historical test results over a sufficiently broad period of time. Projected failure rates must be weighed against the benefit of this increased stringency.

Subsequent to assay validation, the internal control cut-off value for NHP samples was lowered from 0.500 to 0.300. A retrospective review of data over a 6-month period supported the lower internal control cut-off value. Data from all samples testing negative with an internal control value between 0.300 and 0.500 was reviewed and in each case, the sample tested negative with an internal control cut-off value >0.500 upon repeat. Decreasing the internal control cut-off value from 0.500 to 0.300 reduces the number of invalid test results without any associated risk of not detecting a Parvovirus B19 positive sample.

16. Appendix F

16.1 Justification for Testing Mini-Pools of 96 to 480 Plasma Donations

The *Method for the Detection of Parvovirus B19 DNA in Donation Mini-Plasma Pools using Polymerase Chain Reaction Methodology, Version 2,* can be performed on mini-pools that range in size from 96 to 480 plasma donations. The increased mini-pool size was validated and implemented in 2005 to improve work efficiencies. By decreasing the clinical sensitivity (due to increased sample dilution) processing times increase and unnecessary deconstructions are significantly reduced. There was no change in the validated analytical sensitivity of the assay. Testing mini-pools of up to 480 plasma donations continues to provide a 2-$\log_{10}$ margin of safety below the plasma manufacturing pool specification of 1×10^5 IU/mL B19 DNA.

Testing plasma donation mini-pools in the range of 96 to 480 donations using the existing qualitative test method is an interim measure. A quantitative method for the detection of Parvovirus B19 DNA in plasma donation mini-pools is currently under development and will be validated in accordance with PA/PH/OMCL (03) 38, DEF OMCL Guideline For Validation of Nucleic Acid Amplification Techniques (NAT) for Quantitation of B19 Virus DNA in Plasma Pools.

Talecris Biotherapeutics, Inc.
Clayton, NC

T.18.47-01

Validation of the Parvovirus B19 Fluorogenic Donation Qualification Assay, Version 1 (B19 FDQA, v1), NAT Development

Validation of Test Methods

Number of Pages
(including cover page)

46

Original is signed

Name and Rank Date

Name and Rank Date

Valid from: 16 Jul 2007

Table of Contents

1. Introduction

Human parvovirus B19 is the single member of the genus Erythrovirus in the Parvoviridae family. It is a non-enveloped, single stranded DNA virus that can cause symptoms with clinical relevance. Parvovirus B19 is the etiologic agent of the childhood illness erythema infectiosum (a.k.a. Fifth Disease), acute anemia resulting from transient aplastic crisis, and hydrops fetalis during early pregnancy. The two groups of people most susceptible to parvovirus B19 infection are immune compromised individuals and pregnant women. Parvovirus B19 infection is highly contagious, primarily through the respiratory route. However, infection may be transmitted parenterally through the infusion of blood or blood-derived products. Parvovirus B19 is resistant to traditional inactivation steps, such as pasteurization and treatment with detergent or solvent.

The parvovirus B19 Fluorogenic Qualification Assay, version 1 (B19 FDQA, v1) is an assay developed at Talecris Biotherapeutics for the detection of parvovirus B19 in human plasma. The B19 FDQA, v1 is a quantitative test with the sample results reported with a specific concentration in International Units per milliliter (IU/mL) by calibration to the 1st WHO International Standard for parvovirus B19 DNA NAT assays, 99/800. This assay is being implemented by Talecris Biotherapeutics as a part of the overall safety strategy for the manufacture of therapeutic protein products. Regulatory agencies currently do not require parvovirus B19 testing of plasma used to manufacture plasma protein therapeutics. The FDA has allowed NAT testing of plasma pools for parvovirus B19 as an "in-process" test to ensure the quality of plasma for further manufacture. The critical control point at Talecris is parvovirus B19 testing of the plasma manufacturing pool since the goal of an "in-process" test is to assure the levels of parvovirus B19 in the plasma manufacturing pool meet Talecris specifications. The B19 FDQA, v1 functions as a quantitative test for impurities' content.[1]

The B19 FDQA, v1 is comprised of extraction and purification of parvovirus B19 DNA from human plasma followed by real-time polymerase chain reaction (PCR) amplification of a conserved 147 base pair region of the parvovirus B19 genome encoding the nonstructural 1 (NS1) protein with simultaneous detection of the PCR product by cleavage of dual-labeled fluorescent oligonucleotide probes specific to the amplified 5' non-translated target region (real-time detection).

The chaotropic detergent cetyltrimethylammonium bromide (CTAB) in combination with Roche AmpliScreen Multiprep Sample Prep reagents (guanidine isothiocyanate (GITC) buffer)[2] are used to extract parvovirus B19 DNA from human plasma. The recovered DNA is amplified with AmpliTaq Gold DNA Polymerase in the Applied Biosystems

[1] ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology. Current Step 4 version Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005)
[2] AmpliScreen HIV-1Test, v 1.5 draft procedure, Roche Molecular Systems, Inc., Somerville, NJ.

TaqMan® 2X Universal PCR Master Mix[3] using the 5' forward primer B19 19A3 and the 3' reverse primer B19 19A4U.

An internal control (IC), B19 IC4a, is included with each sample tested. B19 IC4a consists of a DNA sequence unrelated to known blood borne pathogens flanked by DNA sequences complementary to the B19 19A3 and B19 19A4U primers used to detect the parvovirus B19 target in the B19 FDQA, v1. The B19 IC4a DNA IC is added during the sample extraction procedure after the addition of the CTAB solution to distinguish true negative results from false negative results due to nucleic acid recovery, sample transfer, amplification, and/or detection.

Parvovirus B19 DNA and IC DNA are amplified concurrently and competitively since both target and IC contain B19 19A3 and B19 19A4U primer binding sequences. The amplified products are detected by fluorescent signals generated during each cycle of PCR amplification, hence real-time detection.

The B19 FDQA, v1 utilizes three fluorogenic different probes. The first probe, the B19 Wild Type Probe (B19WTTA), detects parvovirus B19 Genotype 1. The second probe, the B19 Universal Variant Probe (B19UVPAC), detects the parvovirus B19 target sequences from parvovirus B19 Genotype 2 (A6) and Genotype 3 (V9 and D91.1). Both these dual-labeled probes contain a fluorescent dye, FAM (carboxyfluorescein), at the 5' end, and a quencher molecule, BHQ-1 (Black Hole Quencher-1), at the 3' end that absorbs energy emitted from the fluorescent dye. The probe is cleaved when the AmpliTaq Gold DNA Polymerase replicates the region where the probe is bound, permitting the FAM dye to fluoresce at a unique wavelength. The same approach detects IC DNA which is detected with the third probe, the IC3825JAC probe, which binds to a unique IC target region between the parvovirus B19 primer binding sites. Amplification of the IC target with the IC3825JAC probe bound cleaves the probe releasing the JOE fluorescent dye (6-carboxy-4', 5'-dichloro-2', 7'-dimethoxy-fluorescein) to fluoresce at its unique wavelength. The different fluorescence emissions by the FAM (parvovirus B19 target) and JOE (IC) dyes are detected concurrently on the AB7300 Real-Time PCR System® permitting the parvovirus B19 and IC amplifications to be detected differentially.

Talecris will test manufacturing pools, the control point for viral load determination, to ensure that the manufacturing pools meet Talecris specifications. To ensure the conformance of manufacturing pools, Talecris will test plasma sample masterpools of 384 to 480 donations using the B19 FDQA, v1 to identify and interdict donations containing elevated levels of parvovirus B19.

[3] Package insert for Applied Biosystems TaqMan® 2X Universal PCR Master Mix.

2. Objective

The aim of this study was to validate the parvovirus B19 Fluorogenic Donation Qualification Assay, version 1 (B19 FDQA, V.1). The validation protocol was performed according to the ICH guideline for Validation of Analytical Procedures[1] and the OMCL Guideline for Validation of Nucleic Acid Amplification Techniques (NAT) For Quantitation of parvovirus B19 DNA in Plasma Pools, 2005.[4] The methods described in the attendant procedures provide the basis for testing plasma manufacturing pools and testing plasma sample masterpools that represent 384 to 480 plasma donations for parvovirus B19. This method is also applicable to the resolution and confirmatory phases of testing for parvovirus B19 elevated pools, where plasma sample pools representing either 96, 12 or 8 plasma donations or individual plasma donations are tested.

Specifically, the validation protocol was designed to address the application of the B19 FDQA, v1 which includes 1.0 mL CTAB/GITC nucleic acid extraction procedure coupled with amplification and simultaneous detection of parvovirus B19 target and IC sequences using the B19 19A3 and B19 19A4U primers and specific target probes B19WTTA, B19UVPAC and IC3825JAC. The AB7300 Real-Time PCR instrument concurrently monitors fluorescence from these dual-labeled probes during each amplification cycle. An increase in the appropriate fluorescence signal over time indicates that parvovirus B19 target and/or IC is present in the test sample.

3. Testing Facility

Talecris Biotherapeutics, Inc.
Raleigh Test Laboratory
Nucleic Acid Technology Development Laboratory
1200 New Hope Road
Raleigh, North Carolina 27610
(919) 250-5184

4. Persons Involved in the Study

Study Director:
Anne Keen, Research Scientist

Study Personnel:

Martesa Williams, Senior Associate Research Scientist	Analyst 1
Leslie Tremlett, Senior Associate Scientist II	Analyst 2
Beth Lonesky, Senior Associate Research Scientist	Analyst 3
Joshua Marley, Associate Research Scientist	Analyst 4
Kevin Sullivan, Senior Associate Scientist II	Analyst 5

[4] OMCL Guideline for Validation of Nucleic Acid Amplification Techniques (NAT) For Quantitation of B19 Virus DNA in Plasma Pools, European Directorate for the Quality of Medicines (EDQM), Control Authority Batch Release of Blood Products, 2005.

Justin Paul, Associate Research Scientist	Analyst 6
Holly Vestal, Associate Research Scientist	Analyst 7

5. Dates of Study

Initiated: 02 March 2007

Completed: 23 March 2007

6. Description of Materials Tested

B19 In-house Standard, NAT-126

The Talecris B19 In house Standard, NAT-126, was used. NAT-126 is a parvovirus B19-positive plasma donation that tested non-reactive for HIV-1, HCV, and HBV using PCR methodologies. The Talecris parvovirus B19 In-house Standard was calibrated against the 1st WHO International Standard for parvovirus B19 DNA NAT assays, 99/800[5] and the titer was determined to be 2.7×10^{11} IU/mL.

HBV In-house Standard, NAT-098

The HBV in house standard, NAT-098, is high titer HBV-positive human serum that was separated from the clot and frozen at -70°C within four hours of collection. NAT-098 tested non-reactive for HAV, HIV-1, HBV, and parvovirus B19 using PCR methodologies. The HBV In-house Standard was calibrated relative to the WHO International Standard for HBV DNA NAT Assays, 97/746. The titer was determined to be 7.6×10^{8} IU/mL.

Negative Control and Sample Panel Diluent

A multiple-source plasma pool, NAT-129, that tested negative for HIV-1, HCV, HBV, HAV, and parvovirus B19 using PCR methodologies was used as diluent (NHP diluent) for the preparation of the negative diluent controls, preparation of viral intermediates, and preparation of test panels that contained parvovirus B19 (Appendix A).

Parvovirus B19 pYT104-C Plasmid Standards

Parvovirus B19 plasmid pYT104-C, containing the B19-Au genome (GenBank accession # M131780), was determined to have a concentration of 0.74×10^{11} copies/µL by PicoGreen quantitation. Since this plasmid is double-stranded DNA, the actual B19 copy number is 1.48×10^{11} copies/µL. For donation testing with the B19 FDQA, v1, pYT104-C was utilized to prepare B19 FDQA, v1 Quantitation Standards B19 QS1 through B19 QS5 ranging from 1.0×10^{5} copies/reaction to 100 copies/reaction. To assess the upper

[5]Saldanha, J., Lelie, N., Yu, M.W. and Heath, A. (2002) Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. Vox Sanguinis 82 (1), 24-31

range of quantitation for manufacturing pool testing with the B19 FDQA, pYT104-C was also utilized to prepare Parvovirus B19 Quantitative Fluorogenic Assay plasmid standards P1 through P8 ranging from 32 copies/reaction to 1.0×10^7 copies/reaction.

Test Sample Panels

The dilution scheme for and the composition of the all test sample panels tested during this validation study are outlined in Appendix A.

7. Analytical Test Method

7.1 Method Description

7.1.1 Extraction

The B19 FDQA, v1 uses the chaotropic detergent cetyltrimethylammonium bromide (CTAB) which is added to the plasma test sample to aid the disruption of viral particles and the recovery of nucleic acids. After a low speed spin, the pellet containing the viral nucleic acid is disrupted with a guanidine isothiocyanate (GITC) buffer that also contains a PolyAcryl carrier co-precipitant to enhance nucleic acid recovery during isopropanol precipitation.

7.1.2 Real-time PCR Amplification

Parvovirus B19 detection by real-time PCR is based on amplification of a specific region of the parvovirus B19 genome and fluorescence detection via fluorescent dyes (fluorophores). The B19 FDQA, v1 targets a conserved region of the genome encoding for the nonstructural 1 (NS1) protein. This region is amplified using Taq DNA polymerase with the 5' forward primer B19 19A3 and the 3' reverse primer B19 19A4U.

The same approach amplifies the internal control (IC) DNA that serves to distinguish true negative results from false negative results caused by extraction errors or amplification and detection inhibition. IC DNA is added to the test sample after the addition of CTAB and serves as a control for nucleic acid recovery, sample transfer, amplification, and detection for each individually processed sample. The IC DNA, which has primer binding sites complementary to the parvovirus B19 5' forward and 3' reverse primers, is amplified concurrently and competitively along with any parvovirus B19 DNA in the test sample.

7.1.3 Fluorogenic Detection

The B19 FDQA, v1 utilizes three different detection probes. The first fluorogenic probe, B19WTTA, detects wild type parvovirus B19. The second fluorogenic probe, B19 Universal Variant Probe, B19UVPAC, detects Genotype 2 (A6) and Genotype 3 (V9 and D91.1). The B19WTTA and B19UVPAC probes anneal specifically between the 5' forward primer, B19 19A3, and the 3' reverse primer, B19 19A4U. Each probe is labeled at the 5' end with the fluorescent dye, FAM, and at the 3' end with the quencher, BHQ-1, that absorbs energy emitted from the fluorescent dye. When the Taq DNA polymerase

amplifies the parvovirus B19 target region with the probe bound, the probe is cleaved permitting the FAM dye to fluoresce at a unique wavelength. This increase in sample fluorescence at a specific wavelength is monitored during the amplification.

The IC DNA is detected with an oligonucleotide probe, IC3825JAC that binds to a unique IC target region between the parvovirus B19 primer binding sites present on the IC DNA. Amplification of the IC target with the IC probe bound cleaves the probe releasing the JOE dye to fluoresce at its unique wavelength. The different emission wavelengths of the FAM (parvovirus B19 target) and JOE (IC) fluorescent dyes are simultaneously detected by the AB7300 Real-Time PCR System® permitting the parvovirus B19 and IC amplifications to be detected differentially.

The increase in both FAM (parvovirus B19 target signal) and JOE (IC signal) fluorescence is measured during each amplification cycle. The cycle number (may be fractional) at which the amplitude of the FAM or JOE signal crosses an established threshold is called the cycle threshold or C_T value. The lower the C_T value, the more parvovirus B19 target analyte is present in a sample. The PCR amplification products are detected by signal generation during each cycle of replication, hence real-time detection.

The B19 FDQA, v1 test is a quantitative test with the test samples reported as B19 non-elevated or with a specific concentration in IU/mL. Plotting the log of initial parvovirus B19 target copy number for the B19 quantitation standards containing a known concentration of the parvovirus B19 plasmid pYT104-C (plus 100 copies IC4a) versus the C_T generates a standard curve. Quantitation of the amount of parvovirus B19 target in each unknown test sample is accomplished by measuring C_T and comparing it to the standard curve to determine the starting parvovirus B19 copy number. The process of calculating FAM C_Ts, preparing a standard curve, and determining starting copy number for unknowns is performed by the AB7300 SDS software. Similarly, any sample that has its IC amplification plot cross a fixed threshold with a valid C_T value is reported as IC positive.

7.2 Quantitation Controls

A set of external quantitation standards of known concentration is included with each test run. The quantitation standards are dilutions of the parvovirus B19 plasmid pYT-104C and also contain 100 copies of IC4a. The plasmid contains the complete parvovirus B19 genome and is amplified and detected using the same primers as the parvovirus B19 target DNA.

The cycle number at which the fluorescence intensity rises above baseline (CT) is determined for each of the plasmid dilutions. A plot of the log of initial target copy number for the plasmid dilution set versus CT is a straight line, the standard curve.

The B19 FDQA, v1 is used in two different configurations depending of the type of samples tested.

Quantitation standards B19 QS1 through B19 QS5 are used to generate the standard curve for donation testing and range from 100 copies/reaction to 1.0×10^5 copies/reaction. The assay runs to assess the B19 FDQA, v1 for donation testing are valid if QS1 yielded a B19 Viral Target C_T between 21.90 and 25.50 with R^2 statistic (coefficient of determination) ≥ 0.980 for the entire QS standard curve.

Quantitation standards B19 P1 through P8 are used to generate the standard curve for manufacturing pool testing and range from 32 copies/reaction to 1.0×10^7 copies/reaction. The assay runs to assess the B19 FDQA, v1 for manufacturing pool testing are valid if P1 yielded a B19 Viral Target C_T between 33.37 to 39.45; P2 C_T between 32.26 to 36.67; P3 C_T between 30.95 to 34.62; P4 C_T between 29.71 to 32.25; P5 C_T between 26.78 to 28.73; P6 C_T between 23.62 to 24.86; P7 C_T between 20.02 to 21.53; and P8 C_T between 16.93 to 17.81.

Quantitation of the amount of target in each test sample is accomplished by measuring CT and comparing it to the standard curve to determine the initial parvovirus B19 copy number. The process of calculating FAM C_T values, preparing the standard curve, and determining starting copy number for test samples is performed by the AB7300 SDS software.

7.3 Assay Validation Controls

Assay runs are deemed valid by the appropriate performance of quantitation standards and negative, positive, and internal controls.

One negative control (NEG), an aliquot of NHP containing IC4a, is extracted, amplified, and detected in each assay run to ensure the amplification reagents are not contaminated with target and to verify the performance of the internal control.

Two positive control samples are extracted, amplified, and detected with each assay run to measure the performance of each process within the assay. The B19 low positive control, B19 LOW, contains 1.0×10^3 IU/mL B19; the B19 high positive control, B19 HIGH, contains 1.0×10^5 IU/mL B19. The calculated parvovirus B19 titer for the B19 LOW and HIGH controls is required to be within 4-fold of the input titer (IU/mL).

Therefore, each assay run of the B19 FDQA, v1 is valid if the NEG tests B19 negative; the B19 LOW yields a quantitative value between 2.50×10^2 IU/mL and 4.00×10^3 IU/mL B19; and the B19 HIGH yields a quantitative value between 2.50×10^4 IU/mL and 4.00×10^5 IU/mL.

Once an assay run is ruled valid, the test samples are interpreted as to their concentration of parvovirus B19 in IU/mL, B19 non-elevated (NEL), or invalid.

A parvovirus B19 variant trending control, the B19 VTC, is also included in each assay run. The B19 VTC contains 1.00×10^4 copies of a synthetic plasmid containing the target region from parvovirus B19 Genotype 2 (A6). The B19 VTC is used as a positive

amplification control for B19 variant detection. The B19 VTC result is not interpreted, but was included for information purposes only.

7.4 Test Disposition

Once the assay run is determined to be valid, the disposition of each sample is determined. Briefly, the parvovirus B19 quantitation is determined for a test sample based on any target C_T value < 38.00 and the standard curve generated from the external quantitation standards. Since the IC competes for the amplification primers, the IC is not used to deem a test sample positive for parvovirus B19. The IC is used to deem a test sample negative for parvovirus B19 if the parvovirus B19 target C_T is ≥ 38.00 and the IC C_T is between 32.00 and 38.50.

If a test sample does not meet these acceptance criteria as either positive for parvovirus B19 with a valid quantitative result or negative for parvovirus B19 with a valid IC result, the sample result is deemed invalid and the sample is retested.

8. Design of the Validation Study

The B19 FDQA, v1 described here functions as a quantitative test for impurities. Test results should accurately reflect the amount of a detectable analyte in a sample. Therefore, the parameters most important for validation of the B19 FDQA, v1 are specificity, linearity, accuracy, limit of detection, limit of quantitation, range, and precision. In addition, this validation assessed several robustness aspects that could affect the ability of the B19 FDQA, v1 to accurately quantify parvovirus B19 in plasma.

The elements of the validation study design and acceptance criteria are summarized in Table 1.

Table 1. Design and Acceptance Criteria used for the Validation of the Parvovirus B19 Fluorogenic Donation Qualification Assay, version 1 (HB19 FDQA, v1)

Validation Parameter	Test Samples	Number of Operators	Number of Samples / Operator	Acceptance Criteria
Specificity	Negative diluent controls: plasma donations	1	20	100% negative (FAM $C_T \geq 38.00$)
Specificity	Negative diluent controls: plasma initial combined samples	1	20	100% negative (FAM $C_T \geq 38.00$)
Specificity	Negative diluent controls: Plasma masterpools	1	20	100% negative (FAM $C_T \geq 38.00$)
Specificity	Negative diluent controls: model manufacturing pools	1	20	100% negative (FAM $C_T \geq 38.00$)
Specificity	Negative diluent controls: NHP containing 5.0×10^5 IU/mL HBV	3	8	100% negative (FAM $C_T \geq 38.00$)
Specificity	Test panel: NHP containing 1.0×10^3 IU/mL B19 and 5.0×10^5 IU/mL HBV and NHP containing 1.0×10^5 IU/mL B19 and 5.0×10^7 IU/mL HBV	3	16 (8 at each viral titer)	Mean titer within ± 4-fold of input titer and %CV ≤ 50% collectively
Linearity	Test Panel for B19 FDQA, v1: NHP containing 3.2, 10, 32, 100, 320, 1.0×10^3, 3.2×10^3, 1.0×10^4, and 1.0×10^5 IU/mL B19 Test Panel for manufacturing pool testing: NHP containing 3.2, 10, 32, 100, 320, 1.0×10^3, 3.2×10^3, 1.0×10^4, 1.0×10^5, 1.0×10^6, and 1.0×10^7 IU/mL B19	4	66 (6 at each viral titer)	For B19 FDQA, v1 and manufacturing pool testing: $R^2 \geq 0.980$
Accuracy				For B19 FDQA, v1 and manufacturing pool testing: Collective mean titer within ± 4-fold of input titer
Limit of Detection (LOD)				For B19 FDQA, v1 and manufacturing pool testing: $\leq 2.0 \times 10^2$ IU/mL B19
Limit of Quantitation (LOQ)				For B19 FDQA, v1 and manufacturing pool testing: $\leq 3.2 \times 10^2$ IU/mL B19
Range				For B19 FDQA, v1: Minimum range from 3.2×10^2 IU/mL to 1.0×10^5 IU/mL B19 For manufacturing pool testing: Minimum range from 3.2×10^2 IU/mL to 1.0×10^7 IU/mL B19

Validation Parameter	Test Samples	Number of Operators	Number of Samples / Operator	Acceptance Criteria
Precision: repeatability and intermediate precision	Test panel: NHP containing 1.0×10^3, 1.0×10^5, and 1.0×10^7 IU/mL B19	3	24 (8 at each viral titer)	Repeatability: %CV ≤ 50% for each operator, and Intermediate precision: %CV ≤ 50% collectively
Robustness (at threshold levels)	Test panel: NHP and NHP containing 2.0×10^3, 1.0×10^4, 5.0×10^4, and 1.0×10^5 IU/mL B19	4	25 (5 at each viral titer)	Mean titer within ± 4-fold of input titer and %CV ≤ 50% collectively
Robustness (reagent variability)	Test panel: NHP and NHP containing 2.0×10^3, 1.0×10^4, 5.0×10^4, and 1.0×10^5 IU/mL B19 amplified with 2 different lots of B19 FDQA Master Mix	4	25 (5 at each viral titer)	Mean titer within ± 4-fold of input titer and %CV ≤ 50% collectively for each MMX lot
Robustness (cross-contamination)	Negative diluent control: NHP	1	15	100% non-elevated (NEL) (FAM $C_T \geq 38.00$)
Robustness (cross-contamination)	Test panel: NHP containing 1.0×10^9 IU/mL B19	1	15	100% detection frequency

As a component of assay execution, each validation parameter assessment consisted of separate extraction, amplification, and detection sessions, and each analyst performed each phase of the assay.

For the validation study, B19 FDQA Master Mix (MMX) was prepared and stored at ≤ -20°C until quality control (QC) testing of the B19 FDQA MMX and other reagents was performed.

This validation was designed to assess specificity, linearity, accuracy, limit of detection, limit of quantitation, range, precision, and robustness to confirm that the B19 FDQA, v1, with the 1.0 mL CTAB/GITC nucleic acid extraction method, real-time PCR amplification, and detection is suitable for testing plasma. Individual studies and their acceptance criteria are further defined in the following sections. Test sample panel preparation is outlined in Appendix A.

8.1 Specificity

Specificity is primarily a function of oligonucleotide primer and probe selection as well as the stringency of test conditions in PCR-based assays. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Specificity was evaluated in 2 separate assessments for this validation.

In the first assessment, 4 negative plasma pool matrices were tested to establish that the B19 FDQA, v1 does not generate false-positive reactions from its integral components or pool constituents. Twenty plasma donations, 20 plasma initial combined samples, 20 plasma masterpools, and 20 model manufacturing pools were tested in the B19 FDQA, v1. The test samples representing the negative plasma matrices were pre-screened for the presence of HIV-1, HCV, HBV, HAV, and parvovirus B19 using validated PCR methodologies.

The specificity acceptance criteria of the B19 FDQA, v1 for both donation testing and manufacturing pool testing of the four negative plasma matrices are met if 100% of the samples test negative for parvovirus B19 DNA and generate valid IC results.

In the second specificity assessment, 5.0×10^5 IU/mL HBV was added to 24 NHP samples, 24 samples containing 1000 IU/mL B19, and 24 samples containing 1.0×10^5 IU/mL B19 to demonstrate specificity in the presence of non-targeted DNA. Three operators extracted and tested 8 test samples at each concentration. The acceptance criteria for NHP samples containing HBV are met if 100% of the samples test negative for parvovirus B19 DNA and generate valid IC results.

The specificity acceptance criteria of the B19 FDQA, v1 for both donation testing and manufacturing pool testing of the test samples containing parvovirus B19 and HBV are met if parvovirus B19 DNA is quantitated with suitable accuracy within ± 4-fold of input titer collectively and a %CV ≤ 50% collectively.

8.2 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of the B19 FDQA, v1 for donation testing was assessed at the following concentrations: 3.2, 10, 32, 100, 320, 1.0×10^3, 3.2×10^3, 1.0×10^4, and 1.0×10^5 IU/mL B19. To assess B19 FDQA, v1 suitability for manufacturing pool testing, additional test samples at 1.0×10^6 and 1.0×10^7 IU/mL B19 were included in this evaluation to demonstrate linearity at the upper limit of quantitation. Four operators extracted and tested 6 test samples at each concentration. Each operator carried out 3 experiments to generate 24 test results at each dilution of parvovirus B19. Test samples were analyzed for donation testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 QS1 through B19 QS5. Test samples were analyzed for manufacturing pool testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 plasmid standards P1 through P8.

To meet the linearity acceptance criterion, the R^2 statistic (coefficient of determination) of the B19 FDQA, v1 for donation testing and manufacturing pool testing must be ≥ 0.980.

8.3 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the B19 FDQA, v1 for donation testing and manufacturing pool testing was determined simultaneously with linearity. Test samples were analyzed for donation testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 QS1 through B19 QS5. Test samples were analyzed for manufacturing pool testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 plasmid standards P1 through P8.

To meet the accuracy acceptance criterion, the collective mean observed titer of the B19 FDQA, v1 for donation testing and manufacturing pool testing must be within ± 4-fold of the input titer.

8.4 Limit of Detection

The detection limit (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The B19 FDQA, v1 limit of detection for donation testing and manufacturing pool testing was determined simultaneously with linearity. Test samples were analyzed for donation testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 QS1 through B19 QS5. Test samples were analyzed for manufacturing pool testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 plasmid standards P1 through P8. Test samples with FAM C_T values ≥ 38.00 were considered negative for this portion of the analysis.

To meet the limit of detection acceptance criterion, the limit of detection of the B19 FDQA, v1 for donation testing and manufacturing pool testing must be $\leq 2.0 \times 10^2$ IU/mL B19.

8.5 Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The B19 FDQA, v1 limit of quantitation for donation testing and manufacturing pool testing was determined simultaneously with linearity. Test samples were analyzed for donation testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 QS1 through B19 QS5. Test samples were analyzed for manufacturing pool testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 plasmid standards P1 through P8.

To meet the limit of quantitation acceptance criterion, the limit of quantitation of the B19 FDQA, v1 for donation testing and manufacturing pool testing must be $\leq 3.2 \times 10^2$ IU/mL B19.

8.6 Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the test sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range of the B19 FDQA, v1 for donation testing and manufacturing pool testing was determined simultaneously with linearity. Test samples were analyzed for donation testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 QS1 through B19 QS5. Test samples were analyzed for manufacturing pool testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 plasmid standards P1 through P8.

To meet the range acceptance criterion, the acceptable range of the B19 FDQA, v1 for donation testing must meet or exceed 3.2×10^2 IU/mL to 1.0×10^5 IU/mL B19.

To meet the range acceptance criterion, the acceptable range of the B19 FDQA, v1 for manufacturing pool testing must meet or exceed 3.2×10^2 IU/mL to 1.0×10^7 IU/mL B19.

8.7 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test sample under the prescribed conditions. Precision is validated at two levels: repeatability (the same operating conditions over a short interval of time) and intermediate precision (expresses variations within the laboratory: different days, different operators, and different equipment). Precision of the B19 FDQA, v1 for donation testing was assessed at 1.0×10^3 and 1.0×10^5 IU/mL B19. To assess B19 FDQA, v1 suitability for manufacturing pool testing, precision was also assessed at 1.0×10^7 IU/mL B19 to demonstrate precision at the upper limit of quantitation. Repeatability and intermediate precision were assessed by 3 operators performing the B19 FDQA, v1 on different days using different AB7300 instruments. Each operator extracted and tested 8 samples at each concentration to generate 24 test results at each dilution of parvovirus B19. Test samples were analyzed for donation testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 QS1 through B19 QS5. Test samples were analyzed for manufacturing pool testing with the B19 FDQA, v1 utilizing the standard curve generated from B19 plasmid standards P1 through P8.

To meet the precision acceptance criteria, the B19 FDQA, v1 must quantitate parvovirus B19 DNA for donation testing and manufacturing pool testing with %CV ≤ 50% for each operator to demonstrate acceptable repeatability and %CV ≤ 50% collectively to demonstrate acceptable intermediate precision.

8.8 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an

indication of its reliability during normal usage. Robustness was evaluated in 3 assessments for the validation of the B19 FDQA, v1.

In the first robustness assessment, the B19 FDQA, v1 was tested for its ability to quantitate parvovirus B19 DNA at threshold concentrations used to accept or reject plasma donations to ensure that manufacturing pools meet Talecris specifications. Robustness at threshold concentrations for the B19 FDQA, v1 was assessed in NHP and in test samples at 2.0×10^3, 1.0×10^4, 5.0×10^4, and 1.0×10^5 IU/mL B19. Four operators extracted and tested 5 samples at each concentration to generate 20 test results at each concentration.

To meet the robustness acceptance criteria at threshold concentrations, the B19 FDQA, v1 must accurately detect negative samples with a FAM C_T value $\geq$ 38.00 and a valid IC as well as accurately quantitate samples containing parvovirus B19 DNA with a mean observed titer within $\pm$ 4-fold of the input titer collectively and with %CV $\leq$ 50% collectively.

The second robustness assessment examined the reliability of the B19 FDQA, v1 when variations in reagents occur. Test samples utilized to demonstrate robustness at threshold concentrations were amplified with two additional lots of B19 FDQA Master Mix (MMX) that were different from the B19 FDQA MMX lot utilized throughout the remainder of the validation studies. Each additional B19 FDQA MMX lot contained primers, probes, and MMX which were different from the primers, probes, and MMX lots utilized in the original B19 FDQA MMX lot. Four operators amplified 5 test samples at each threshold concentration with 2 different lots of B19 FDQA MMX to generate 20 test results at each concentration for both B19 FDQA MMX lots. Test samples were amplified within two days post-extraction.

To meet the robustness acceptance criteria for reagent variability, the B19 FDQA, v1 must accurately detect negative samples with a FAM C_T value $\geq$ 38.00 and a valid IC as well as accurately quantitate samples containing parvovirus B19 DNA with a mean observed titer within $\pm$ 4-fold of the input titer collectively and with %CV $\leq$ 50% collectively for each B19 FDQA MMX lot.

In the third robustness assessment, cross-contamination prevention was demonstrated by the ability of the B19 FDQA, v1 to accurately detect alternating NHP samples and high-titer B19 samples. One operator extracted 15 NHP samples alternating with 15 high-titer samples at 1.0×10^9 IU/mL B19. Test samples were amplified in a checkerboard pattern on the reaction plate.

To meet the robustness acceptance criteria for cross-contamination, the B19 FDQA, v1 must correctly detect negative samples (100% negative; FAM $C_T \geq$ 38.00 with a valid IC) and high-titer B19 positive samples (100% positive) arranged in a checkerboard.

8.9 Methods for Statistical Analysis

The mean ($\bar{x}$) is defined as the sum of the values divided by the number of values used.

$$\bar{x} = \frac{\sum x_i}{n}$$

The estimated standard deviation (s_{n-1}) is defined as the square root of the estimation

$$s_{n-1} = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$

of the variance for the source data population.

The variation coefficient (% CV) measures the relative dispersion and is obtained by dividing the estimated standard deviation by the mean and multiplying by 100.

$$\%CV = \left(\frac{S_{n-1}}{\bar{x}} \right) 100$$

The coefficient of determination (R^2) indicates the fraction of the total variability in the data being explained by the fitted model. The R^2 statistic is the square of the correlation coefficient between the predicted Ys and the observed Ys. The R^2 statistic is calculated in Microsoft Excel utilizing the following equation:

$$R^2 = 1 - \frac{SSE}{SST}$$

$$\text{where } SSE = \sum (Y_i - \hat{Y}_i)^2$$

$$\text{and } SST = \sum (Y_i - \bar{Y})^2$$

Probit (P) is a model for binary response data based on the normal distribution function. The analytical expression of the model is as follows:

$$P = F(aX + b)$$

where F is the standard normal cumulative function and a and b are the coefficients optimized using a Newton Raphson algorithm.

If the coefficient of variance (%CV) for a data set is > 50%, Dixon's Q-test for rejection of outliers is applied to determine if a single discrepant value can be removed from the data set.

$$Q_{calc} = \frac{|\text{suspect value} - \text{nearest neighbor}|}{(\text{range of entire data set})} = \frac{|x_1 - x_2|}{(x_n - x_1)} \text{ or } \frac{|x_n - x_{n-1}|}{(x_n - x_{n-1})}$$

where x_1 is the extreme low value (or x_n is the extreme high value) suspected of being an outlier in a data set sorted in ascending order.

If the calculated value of Q, Q_{calc}, is greater than the critical value ($Q_{critical}$) at a given level of confidence, then the suspect value is rejected.[6]

[6] Rorabacher, D.B. (1991) Statistical treatment for rejection of deviant values of Dixon's 'Q' parameter and related subrange ratios at the 95% confidence level. *Anal Chem* **63**, 139-146.

9. Discussion of Experimental Results

This validation was designed to demonstrate that the B19 FDQA, v1 is suitable for its intended use with acceptable specificity, linearity, accuracy, limit of quantitation, range, precision, and robustness. The validation study results are summarized in Table 2. All results shown were obtained from test runs with valid positive, negative, and internal control results.

9.1 Specificity

The results for the first specificity assessment (Table 3) demonstrate that 100% (20/20) of the 20 plasma donations, 20 initial combined samples, 20 plasma masterpools, and 20 model manufacturing pools tested negative for parvovirus B19 DNA.

These results meet the specificity acceptance criteria of the B19 FDQA, v1 for both donation testing and manufacturing pool testing with all negative plasma matrices tested.

The results for the second specificity assessment are summarized in Table 4. For test panels containing NHP and 5.0×10^5 IU/mL HBV, the results demonstrate that 100% (24/24) of samples tested negative for parvovirus B19 DNA with valid IC C_T values. For test panels containing 1.0×10^3 IU/mL B19 and 5.0×10^5 IU/mL HBV, the mean observed B19 titer generated was within ± 4-fold of input titer collectively and the % CV was < 50% collectively. For test panels containing 1.0×10^5 IU/mL B19 and 5.0×10^5 IU/mL HBV, the mean observed B19 titer generated was within ± 4-fold of input titer collectively and the %CV was < 50% collectively.

These results meet the specificity acceptance criterion of the B19 FDQA, v1 for both donation testing and manufacturing pool testing which requires 100% of negative samples containing HBV to test negative for parvovirus B19. The results also meet the specificity acceptance criteria for test samples containing parvovirus B19 and HBV which require parvovirus B19 DNA to be quantitated with suitable accuracy within ± 4-fold of input titer collectively and a %CV ≤ 50% collectively.

9.2 Linearity

The results for the linearity assessment of the B19 FDQA, v1 for donation testing (Table 5 and Figure 1) demonstrate assay linearity based on the R^2 statistic (coefficient of determination) equal to 0.9999 when the calculated observed mean titers are plotted against theoretical input titers.

The results meet the linearity acceptance criterion which requires the R^2 statistic (coefficient of determination) of the B19 FDQA, v1 to be ≥ 0.980 for donation testing.

The results for the linearity assessment of the B19 FDQA, v1 for manufacturing pool testing (Table 6 and Figure 2) demonstrate assay linearity based on the R^2 statistic

(coefficient of determination) equal to 1.000 when the calculated observed mean titers are plotted against theoretical input titers.

The results meet the linearity acceptance criterion which requires the R^2 statistic (coefficient of determination) of the B19 FDQA, v1 to be ≥ 0.980 for manufacturing pool testing.

9.3 Accuracy

The results for the accuracy assessment of the B19 FDQA, v1 for donation testing are summarized in Table 7. Mean observed titers were within ± 4-fold of input titers collectively at 10 IU/mL B19 and above when analyzed with B19 QS1 through B19 QS5 quantitative standards.

The results meet the accuracy acceptance criterion of the B19 FDQA, v1 for donation testing which requires the collective mean observed titer to be within ± 4-fold of the input titer.

The results for the accuracy assessment of the B19 FDQA, v1 for manufacturing pool testing are summarized in Table 8. Mean observed titers were within ± 4-fold of input titers collectively at 32 IU/mL B19 and above when analyzed with B19 plasmid standards P1 through P8.

The results meet the accuracy acceptance criterion of the B19 FDQA,v1 for manufacturing pool testing which requires the collective mean observed titer to be within ± 4-fold of the input titer.

9.4 Limit of Detection

The results for the limit of detection assessment of the B19 FDQA, v1 for donation testing are summarized in Tables 9 and 10. The 95% limit of detection occurs at 17 IU/mL B19 when analyzed with B19 QS1 through B19 QS5 quantitative standards.

The results meet the limit of detection acceptance criterion which requires the B19 FDQA, v1 limit of detection to be $\leq 2.0 \times 10^2$ IU/mL B19 for donation testing.

The results for the limit of detection assessment of the B19 FDQA, v1 for manufacturing pool testing are summarized in Tables 11 and 12. The 95% limit of detection occurs at 58 IU/mL B19 when analyzed with B19 plasmid standards P1 through P8.

The results meet the limit of detection acceptance criterion which requires the B19 FDQA, v1 limit of detection to be $\leq 2.0 \times 10^2$ IU/mL B19 for manufacturing pool testing.

9.5 Limit of Quantitation

The results for the limit of quantitation assessment of the B19 FDQA, v1 for donation testing are summarized in Table 13. Mean observed titers were within ± 4-fold of input

titers collectively at 10 IU/mL B19 and above when analyzed with B19 QS1 through B19 QS5 quantitative standards. The %CVs were < 50% for each operator at 320 IU/mL B19 and above demonstrating suitable repeatability. The %CVs were < 50% collectively at 32 IU/mL B19 and above demonstrating suitable intermediate precision. Overall, the results demonstrate suitable precision at 320 IU/mL B19 and above when analyzed with B19 QS1 through B19 QS5 quantitative standards. Based on accuracy and precision requirements, the limit of quantitation occurs at 320 IU/mL B19 when analyzed with B19 QS1 through B19 QS5 quantitative standards.

The results meet the limit of quantitation acceptance criterion which requires the B19 FDQA, v1 limit of quantitation to be $\leq 3.2 \times 10^2$ IU/mL B19 for donation testing.

The results for the limit of quantitation assessment of the B19 FDQA, v1 for manufacturing pool testing are summarized in Table 14. Mean observed titers were within ± 4-fold of input titers collectively at 32 IU/mL B19 and above when analyzed with B19 plasmid standards P1 through P8. The %CVs were < 50% for each operator at 100 IU/mL B19 and above demonstrating suitable repeatability. The %CVs were < 50% collectively at 32 IU/mL B19 and above demonstrating suitable intermediate precision. Overall, the results demonstrate suitable precision at 100 IU/mL B19 and above when analyzed with B19 plasmid standards P1 through P8. Based on accuracy and precision requirements, the limit of quantitation occurs at 100 IU/mL B19 when analyzed with B19 plasmid standards P1 through P8.

The results meet the limit of quantitation acceptance criterion which requires the B19 FDQA, v1 limit of quantitation to be $\leq 3.2 \times 10^2$ IU/mL B19 for manufacturing pool testing.

9.6 Range

The results for the range assessment of the B19 FDQA, v1 for donation testing are summarized in Table 15. Mean observed titers were within ± 4-fold of input titers collectively at 10 IU/mL B19 and above when analyzed with B19 QS1 through B19 QS5 quantitative standards. The %CVs were < 50% for each operator at 320 IU/mL B19 and above demonstrating suitable repeatability. The %CVs were < 50% collectively at 32 IU/mL B19 and above demonstrating suitable intermediate precision. Overall, the results demonstrate suitable precision at 320 IU/mL and above when analyzed with B19 QS1 through B19 QS5 quantitative standards. The results demonstrate assay linearity based on the R^2 statistic (coefficient of determination) equal to 0.9999 when calculated observed mean titers are plotted against theoretical input titers. Based on accuracy, precision, and linearity requirements, the range is from 320 IU/mL to 1.0×10^5 IU/mL B19 when analyzed with B19 QS1 through B19 QS5 quantitative standards.

The results meet the range acceptance criterion which requires the B19 FDQA, v1 range to meet or exceed 3.2×10^2 IU/mL to 1.0×10^5 IU/mL B19 for donation testing.

The results for the range assessment of the B19 FDQA, v1 for manufacturing pool testing are summarized in Table 16. Mean observed titers were within ± 4-fold of input titers collectively at 32 IU/mL B19 and above when analyzed with B19 plasmid standards P1 through P8. The %CVs were < 50% for each operator at 100 IU/mL B19 and above demonstrating suitable repeatability. The %CVs were < 50% collectively at 32 IU/mL B19 and above demonstrating suitable intermediate precision. Overall, the results demonstrate suitable precision at 100 IU/mL B19 and above when analyzed with B19 plasmid standards P1 through P8. The results demonstrate assay linearity based on the R^2 statistic (coefficient of determination) equal to 1.000 when calculated observed mean titers are plotted against theoretical input titers. Based on accuracy, precision, and linearity requirements, the range is from 100 IU/mL to 1.0×10^7 IU/mL B19 when analyzed with B19 plasmid standards P1 through P8.

The results meet the range acceptance criterion which requires the B19 FDQA, v1 to meet or exceed 3.2×10^2 IU/mL to 1.0×10^7 IU/mL B19 for manufacturing pool testing.

9.7 Precision

The results from the precision assessment of the B19 FDQA, v1 for donation testing are summarized in Table 17. The %CVs were < 50% for each operator at 1.0×10^3 IU/mL and at 1.0×10^5 IU/mL B19 demonstrating suitable repeatability. The %CVs were < 50% collectively at 1.0×10^3 IU/mL and at 1.0×10^5 IU/mL B19 demonstrating suitable intermediate precision. Overall, the results demonstrate suitable precision at both concentrations tested when analyzed with B19 QS1 through B19 QS5 quantitative standards.

The results meet the precision acceptance criteria which require the B19 FDQA, v1 to quantitate parvovirus B19 DNA with %CV ≤ 50% for each operator to demonstrate acceptable repeatability and %CV ≤ 50% collectively to demonstrate acceptable intermediate precision for donation testing.

The results for the precision assessment of the B19 FDQA, v1 for manufacturing pool testing are summarized in Table 18. The %CVs were < 50% for each operator at 1.0×10^3 IU/mL, 1.0×10^5 IU/mL, and 1.0×10^7 IU/mL B19 demonstrating suitable repeatability. The %CVs were < 50% collectively at 1.0×10^3 IU/mL, 1.0×10^5 IU/mL, and 1.0×10^7 IU/mL B19 demonstrating suitable intermediate precision. Overall, the results demonstrate suitable precision at the three concentrations tested when analyzed with B19 plasmid standards P1 through P8.

The results meet the precision acceptance criteria which require the B19 FDQA, v1 to quantitate parvovirus B19 DNA with %CV ≤ 50% for each operator to demonstrate acceptable repeatability and %CV ≤ 50% collectively to demonstrate acceptable intermediate precision for manufacturing pool testing.

9.8 Robustness

The results for the initial robustness assessment (Table 19) of the B19 FDQA, v1 for both donation testing and manufacturing pool testing demonstrate that 100% (20/20) of negative samples tested negative for parvovirus B19 DNA with valid IC C_T values. The mean observed parvovirus B19 titer generated at each concentration was within ± 4-fold of input titer collectively and %CVs were < 50% at each concentration collectively.

The results meet the robustness acceptance criteria of the B19 FDQA, v1 for both donation testing and manufacturing pool testing at threshold concentrations which require the B19 FDQA, v1 to accurately detect negative samples with a FAM C_T value ≥ 38.00 and a valid IC as well as to accurately quantitate samples containing parvovirus B19 DNA with a mean observed titer within ± 4-fold of the input titer collectively and with %CV ≤ 50% collectively.

The results for the second robustness assessment of the B19 FDQA, v1 for both donation testing and manufacturing pool testing with 2 different lots of B19 FDQA MMX (Table 20 and 21) demonstrate that 100% (20/20) of negative samples tested negative for parvovirus B19 DNA with valid IC C_T values in MMX Lot 2 and in MMX Lot 3. The mean observed parvovirus B19 titer generated at each concentration was within ± 4-fold of input titer collectively and %CVs were < 50% at each concentration collectively in MMX Lot 2 and in MMX Lot 3.

The results meet the robustness reagent variability acceptance criteria of the B19 FDQA v1 for both donation testing and manufacturing pool testing which require the B19 FDQA, v1 to accurately detect negative samples with a FAM C_T value ≥ 38.00 and a valid IC in each MMX lot as well as to accurately quantitate samples containing parvovirus B19 DNA with a mean observed titer within ± 4-fold of the input titer collectively and with %CV ≤ 50% collectively in each MMX lot.

The results for the third robustness cross contamination assessment (Table 22) of the B19 FDQA, v1 for both donation testing and manufacturing pool testing demonstrate that 100% (15/15) of negative samples tested negative for parvovirus B19 DNA with valid IC C_T values. The results also demonstrate 100% (15/15) detection for high-titer samples at 1.00×10^9 IU/mL B19 with a mean FAM C_T value of 14.19.

The results meet the robustness cross contamination acceptance criteria of the B19 FDQA, v1 for both donation testing and manufacturing pool testing which require the B19 FDQA, v1 to correctly detect negative samples with a FAM C_T value ≥ 38.00 and a valid IC and to correctly detect high-titer B19 positive samples arranged in a checkerboard pattern.

Table 2. Results from the Validation of the Parvovirus B19 Fluorogenic Donation Qualification Assay, version 1 (B19 FDQA, v1)

Validation Parameter	Test Samples	Number of Operators	Number of Samples / Operator	Acceptance Criteria
Specificity	Negative diluent controls: plasma donations	1	20	Met 20/20 (100%) B19 negative
Specificity	Negative diluent controls: plasma initial combined samples	1	20	Met 20/20 (100%) B19 negative
Specificity	Negative diluent controls: Plasma masterpools	1	20	Met 20/20 (100%) B19 negative
Specificity	Negative diluent controls: model manufacturing pools	1	20	Met 20/20 (100%) B19 negative
Specificity	Negative diluent controls: NHP containing 5.0×10^5 IU/mL HBV	3	8	Met 24/24 (100%) B19 negative
Specificity	Test panel: NHP containing 1.0×10^3 IU/mL B19 and 5.0×10^5 IU/mL HBV and NHP containing 1.0×10^5 IU/mL B19 and 5.0×10^5 IU/mL HBV	3	16 (8 at each viral titer)	Met Mean titer (n = 24) within ± 4-fold of input titer and %CV ≤ 50% collectively
Linearity	Test Panel for B19 FDQA, v1: NHP containing 3.2, 10, 32, 100, 320, 1.0×10^3, 3.2×10^3, 1.0×10^4, and 1.0×10^5 IU/mL B19 Test Panel for manufacturing pool testing: NHP containing 3.2, 10, 32, 100, 320, 1.0×10^3, 3.2×10^3, 1.0×10^4, 1.0×10^5, 1.0×10^6, and 1.0×10^7 IU/mL B19	4	66 (6 at each viral titer)	Met For B19 FDQA, v1 and manufacturing pool testing: $R^2 \geq 0.980$
Accuracy				Met For B19 FDQA, v1 and manufacturing pool testing: Collective mean titer within ± 4-fold of input titer
Limit of Detection (LOD)				Met For B19 FDQA, v1 and manufacturing pool testing: LOD ≤ 2.0×10^2 IU/mL B19
Limit of Quantitation (LOQ)				Met For B19 FDQA, v1 and manufacturing pool testing: LOQ ≤ 3.2×10^2 IU/mL B19
Range				Met For B19 FDQA, v1: Minimum range from 3.2×10^2 IU/mL to 1.0×10^5 IU/mL B19

Validation Parameter	Test Samples	Number of Operators	Number of Samples / Operator	Acceptance Criteria
				For manufacturing pool testing: Minimum range from 3.2×10^2 IU/mL to 1.0×10^7 IU/mL B19
Precision: repeatability and intermediate precision	Test panel: NHP containing 1.0×10^3, 1.0×10^5, and 1.0×10^7 IU/mL B19	3	24 (8 at each viral titer)	Met Repeatability: %CV ≤ 50% for each operator and Intermediate precision: %CV ≤ 50% collectively
Robustness (at threshold levels)	Test panel: NHP and NHP containing 2.0×10^3, 1.0×10^4, 5.0×10^4, and 1.0×10^5 IU/mL B19	4	25 (5 at each viral titer)	Met Mean titer within ± 4-fold of input titer and %CV ≤ 50% collectively
Robustness (reagent variability)	Test panel: NHP and NHP containing 2.0×10^3, 1.0×10^4, 5.0×10^4, and 1.0×10^5 IU/mL B19 amplified with 2 different lots of B19 FDQA Master Mix	4	25 (5 at each viral titer)	Met Mean titer within ± 4-fold of input titer and %CV ≤ 50% collectively for each MMX lot
Robustness (cross-contamination)	Negative diluent control: NHP	1	15	Met 15/15 (100%) B19 negative
Robustness (cross-contamination)	Test panel: NHP containing 1.0×10^9 IU/mL B19	1	15	Met 15/15 (100%) B19 positive

10. Conclusions

These studies verify that the B19 FDQA, v1 using real-time polymerase chain reaction methodology consistently quantifies parvovirus B19 in complex matrices and in the presence of a contaminating DNA virus, HBV. The linearity, accuracy, limit of detection, limit of quantitation, and range results were achieved by multiple operators assessing parvovirus B19 quantitation over a range of parvovirus B19 concentrations. The precision results were achieved by multiple operators assessing parvovirus B19 concentrations at the upper and lower limits of the assay on different days using separate instruments. Robustness was demonstrated by quantifying parvovirus B19 at threshold concentrations with multiple lots of B19 FDQA MMX and when extracting and amplifying alternating NHP and high-titer parvovirus B19 test samples.

These studies verified that the B19 FDQA, v1 is suitable for its intended use to quantitate parvovirus B19 in plasma manufacturing pools and testing plasma sample masterpools that represent 384 to 480 plasma donations. This method is also an appropriate method for the resolution and confirmatory phases of testing for parvovirus B19 elevated pools, where plasma sample pools representing 96, 12, or 8 plasma donations or individual plasma donations are tested.

Table 3. B19 FDQA, v1 validation results for specificity of negative plasma matrices

Sample	Number / Total B19 positive	% Positive	Number / Total IC Positive	IC C_T Average	IC C_T Range
Plasma donations	0 / 20	0	20 / 20	35.11	33.89 to 36.28
Initial combined	0 / 20	0	20 / 20	34.96	34.16 to 37.24
Plasma Masterpools	0 / 20	0	20 / 20	34.97	33.42 to 36.56
Model Manufacturing pools	0 / 20	0	20 / 20	34.55	33.67 to 35.53

Table 4. B19 FDQA, v1 validation results for specificity using samples containing 5.0×10^5 IU/mL HBV

Sample Name	Number / Total B19 positive	% Positive	Mean Observed B19 Titer (IU/mL)	Std. Dev.	% CV	IC C_T Average	IC C_T Range
NHP + 5.0×10^3 IU/mL HBV	0/24	0	N/A	N/A	N/A	34.90	33.90 to 35.83
1.0×10^3 IU/mL B19 + 5.0×10^5 IU/mL HBV	24/24	100	3.4×10^3	6.6×10^2	19.6%	35.71	N/A
1.0×10^5 IU/mL B19 + 5.0×10^5 IU/mL HBV	24/24	100	3.1×10^5	6.5×10^4	21.3%	N/A	N/A

Table 5. B19 FDQA, v1 linearity results for donation testing: mean, estimated standard deviation, and %CV for data analyzed with B19 QS1 through B19 QS5

Parvovirus B19 Input titer (IU/mL)	# detected samples	Mean Observed Parvovirus B19 titer (IU/mL)	Std. Dev. Total	%CV Total
3.2	13/24	2.1×10^1	4.8×10^1	230.7%
10	18/24	3.2×10^1	2.8×10^1	85.2%
32	24/24	1.1×10^2	3.7×10^1	34.9%
100	24/24	3.5×10^2	1.5×10^2	42.1%
320	24/24	1.0×10^3	2.6×10^2	25.6%
1.0×10^3	24/24	3.2×10^3	5.1×10^2	15.8%
3.2×10^3	24/24	9.8×10^3	1.7×10^3	17.3%
1.0×10^4	24/24	2.8×10^4	4.5×10^3	15.9%
1.0×10^5	24/24	2.5×10^5	4.5×10^4	17.9%

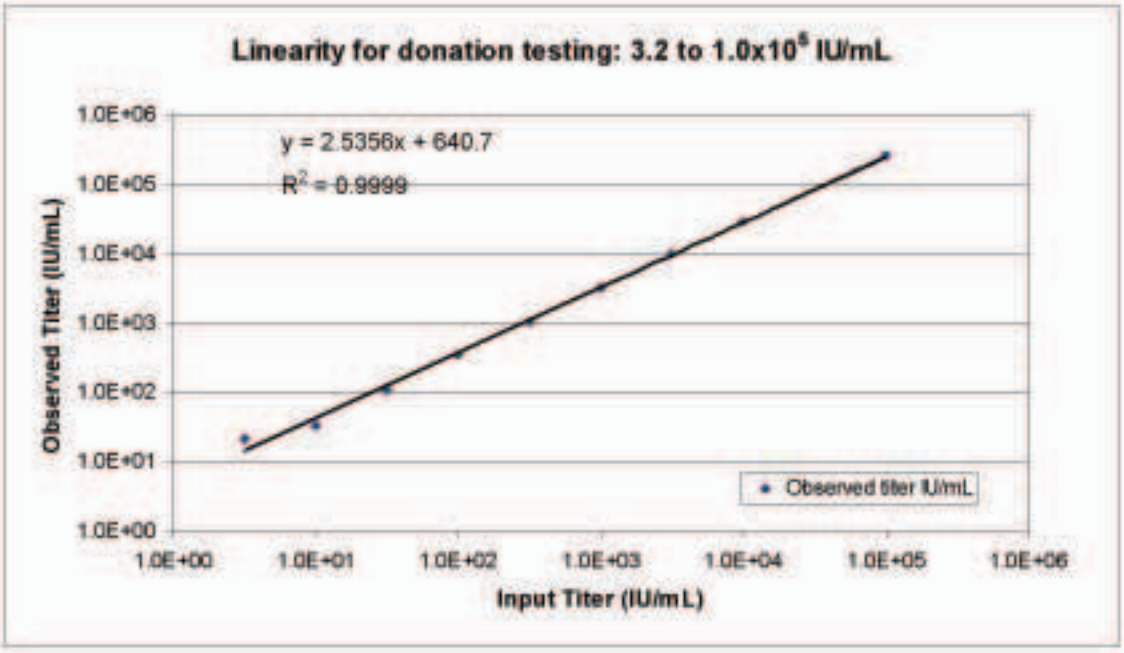

Figure 1 Graph showing the linear relationship across the range of parvovirus B19 titers using NAT-126. Plasma samples containing 3.2 IU/mL to 1.0×10^5 IU/mL B19 were quantified using the B19 FDQA, v1 with quantitation standards B19 QS1 through B19 QS5. The experimentally measured titers of dilutions were plotted against the known input titer to demonstrate linearity across the range of titers. The coefficient of determination (R^2) was calculated to be 0.9999, which meets acceptance criteria of ≥ 0.980.

Table 6. B19 FDQA, v1 linearity results for manufacturing pool testing: mean, estimated standard deviation, and %CV for data analyzed with P1 through P8

Parvovirus B19 Input titer (IU/mL)	# detected samples	Mean Observed Parvovirus B19 titer (IU/mL)	Std. Dev. Total	%CV Total
3.2	13/24	2.1×10^{1}	3.5×10^{1}	169.9%
10	18/24	4.1×10^{1}	3.1×10^{1}	76.1%
32	24/24	1.2×10^{2}	5.0×10^{1}	42.8%
100	24/24	3.5×10^{2}	1.2×10^{2}	34.1%
320	24/24	1.1×10^{3}	4.6×10^{2}	39.8%
1.0×10^{3}	24/24	3.5×10^{3}	9.1×10^{2}	25.7%
3.2×10^{3}	24/24	1.1×10^{4}	2.5×10^{3}	23.3%
1.0×10^{4}	24/24	3.1×10^{4}	7.6×10^{3}	24.5%
1.0×10^{5}	24/24	2.9×10^{5}	8.5×10^{4}	29.4%
1.0×10^{6}	24/24	3.0×10^{6}	8.4×10^{5}	27.6%
1.0×10^{7}	24/24	3.0×10^{7}	6.9×10^{6}	22.7%

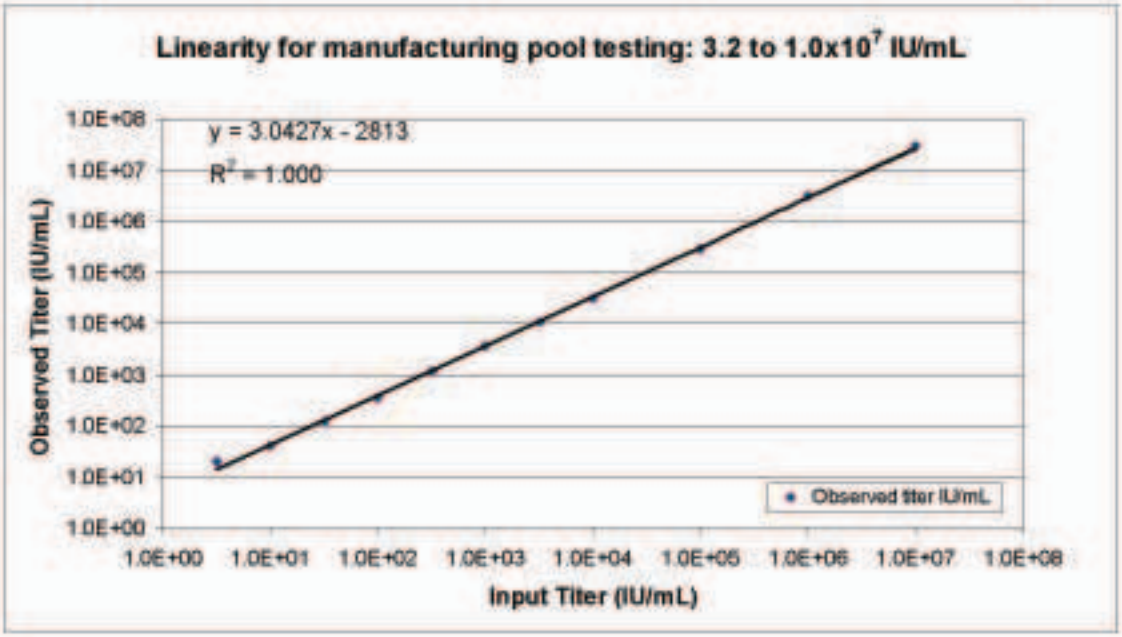

Figure 2. Graph showing the linear relationship across the range of parvovirus B19 titers using NAT-126. Plasma samples containing 3.2 IU/mL to 1.0×10^{7} IU/mL B19 were quantified using the B19 FDQA, v1 with B19 plasmid standards P1 through P8. The experimentally measured titers of dilutions were plotted against the known input titer to demonstrate linearity across the range of titers. The coefficient of determination (R^2) was calculated to be 1, which meets acceptance criteria of ≥ 0.980.

Table 7. B19 FDQA, v1 accuracy results for donation testing: data analyzed with B19 QS1 through B19 QS5

Parvovirus B19 Input titer (IU/mL)	Acceptable Variation -- Input value ± 4-fold (IU/mL)		Mean Observed Parvovirus B19 titer (IU/mL)	In range
3.2	8.0×10^{-1}	1.3×10^{1}	2.1×10^{1}	n
10	2.5×10^{0}	4.0×10^{1}	3.2×10^{1}	y
32	8.0×10^{0}	1.3×10^{2}	1.1×10^{2}	y
100	2.5×10^{1}	4.0×10^{2}	3.5×10^{2}	y
320	8.0×10^{1}	1.3×10^{3}	1.0×10^{3}	y
1.0×10^{3}	2.5×10^{2}	4.0×10^{3}	3.2×10^{3}	y
3.2×10^{3}	8.0×10^{2}	1.3×10^{4}	9.8×10^{3}	y
1.0×10^{4}	2.5×10^{3}	4.0×10^{4}	2.8×10^{4}	y
1.0×10^{5}	2.5×10^{4}	4.0×10^{5}	2.5×10^{5}	y

Table 8. B19 FDQA, v1 accuracy results for manufacturing pool testing: data analyzed with P1 through P8

Parvovirus B19 Input titer (IU/mL)	Acceptable Variation -- Input value +/- 4-fold (IU/mL)		Mean Observed Parvovirus B19 titer (IU/mL)	In range
3.2	8.0×10^{-1}	1.3×10^{1}	2.1×10^{1}	n
10	2.5×10^{0}	4.0×10^{1}	4.1×10^{1}	n
32	8.0×10^{0}	1.3×10^{2}	1.2×10^{2}	y
100	2.5×10^{1}	4.0×10^{2}	3.5×10^{2}	y
320	8.0×10^{1}	1.3×10^{3}	1.1×10^{3}	y
1.0×10^{3}	2.5×10^{2}	4.0×10^{3}	3.5×10^{3}	y
3.2×10^{3}	8.0×10^{2}	1.3×10^{4}	1.1×10^{4}	y
1.0×10^{4}	2.5×10^{3}	4.0×10^{4}	3.1×10^{4}	y
1.0×10^{5}	2.5×10^{4}	4.0×10^{5}	2.9×10^{5}	y
1.0×10^{6}	2.5×10^{5}	4.0×10^{6}	3.0×10^{6}	y
1.0×10^{7}	2.5×10^{6}	4.0×10^{7}	3.0×10^{7}	y

Table 9. B19 FDQA, v1 test results for donation testing scored in binary mode for probit analysis: data analyzed with B19 QS1 through B19 QS5

Parvovirus B19 Input titer (IU/mL)	# positive (FAM C_T < 38.00)	# tested	% positive
3.2	1	24	4.2%
10	11	24	45.8%
32	24	24	100.0%
100	24	24	100.0%
320	24	24	100.0%
1.0×10^3	24	24	100.0%
3.2×10^3	24	24	100.0%
1.0×10^4	24	24	100.0%
1.0×10^5	24	24	100.0%

Table 10. B19 FDQA, v1 determination of limit of detection for donation testing

Detection frequency	Parvovirus B19 titer (IU/mL)
1%	0.715
5%	3.563
10%	5.081
20%	6.920
30%	8.246
40%	9.378
50%	10.437
60%	11.496
70%	12.629
80%	13.955
90%	15.793
95%	17.312
99%	20.160

Table 11. B19 FDQA, v1 test results for manufacturing pool testing scored in binary mode for probit analysis: data analyzed with P1 through P8

Parvovirus B19 Input titer (IU/mL)	# positive (FAM C_T < 38.00)	# tested	% positive
3.2	1	24	4.2%
10	12	24	50.0%
32	24	24	100.0%
100	24	24	100.0%
320	24	24	100.0%
1.0×10^3	24	24	100.0%
3.2×10^3	24	24	100.0%
1.0×10^4	24	24	100.0%
1.0×10^5	24	24	100.0%
1.0×10^6	24	24	100.0%
1.0×10^7	24	24	100.0%

Table 12. B19 FDQA, v1 determination of limit of detection for manufacturing pool testing

Detection frequency	Parvovirus B19 titer (IU/mL)
1%	-44.034
5%	-26.507
10%	-17.163
20%	-5.848
30%	2.310
40%	9.281
50%	15.797
60%	22.313
70%	29.284
80%	37.443
90%	48.757
95%	58.101
99%	75.629

Table 13. B19 FDQA, v1 determination of limit of quantitation for donation testing: data analyzed with B19 QS1 through B19 QS5

Parvovirus B19 Input titer (IU/mL)	HV		JMP		JJM		KGS		Collective Data	
	Average IU/mL	% CV	Average IU/mL	% CV	Average IU/mL	% CV	Average IU/mL	% CV	Average IU/mL	% CV
3.2	1.0×10^{1}	111.2%	6.8×10^{0}	163.0%	1.4×10^{1}	102.4%	5.2×10^{1}	177.2%	2.1×10^{1}	230.7%
10	2.6×10^{1}	116.9%	4.0×10^{1}	29.2%	2.1×10^{1}	78.0%	4.2×10^{1}	99.1%	3.2×10^{1}	85.2%
32	1.0×10^{2}	28.0%	1.2×10^{2}	38.6%	8.7×10^{1}	51.4%	1.1×10^{2}	20.2%	1.1×10^{2}	34.9%
100	4.2×10^{2}	52.7%	3.1×10^{2}	33.5%	3.1×10^{2}	32.7%	3.6×10^{2}	38.1%	3.5×10^{2}	42.1%
320	1.0×10^{3}	18.7%	1.0×10^{3}	36.7%	9.7×10^{2}	20.8%	1.0×10^{3}	29.3%	1.0×10^{3}	25.6%
1.0×10^{3}	3.2×10^{3}	17.5%	3.4×10^{3}	10.5%	3.2×10^{3}	21.9%	3.2×10^{3}	15.7%	3.2×10^{3}	15.8%
3.2×10^{3}	1.0×10^{4}	14.2%	9.7×10^{3}	17.6%	8.8×10^{3}	15.7%	1.0×10^{4}	20.2%	9.8×10^{3}	17.3%
1.0×10^{4}	3.1×10^{4}	11.6%	2.9×10^{4}	15.2%	2.3×10^{4}	10.1%	3.0×10^{4}	7.0%	2.8×10^{4}	15.9%
1.0×10^{5}	2.8×10^{5}	15.8%	2.4×10^{5}	17.7%	2.4×10^{5}	19.5%	2.6×10^{5}	16.0%	2.5×10^{5}	17.9%

Table 14. B19 FDQA, v1 determination of limit of quantitation for manufacturing pool testing: data analyzed with P1 through P8

Parvovirus B19 Input titer (IU/mL)	HV		JMP		JJM		KGS		Collective Data	
	Average IU/mL	% CV	Average IU/mL	% CV	Average IU/mL	% CV	Average IU/mL	% CV	Average IU/mL	% CV
3.2	1.7×10^{1}	58.2%	8.6×10^{0}	162.87%	1.4×10^{1}	88.7%	4.3×10^{1}	154.1%	2.1×10^{1}	169.9%
10	5.9×10^{1}	77.4%	4.8×10^{1}	36.30%	2.4×10^{1}	67.9%	3.4×10^{1}	94.1%	4.1×10^{1}	76.1%
32	1.4×10^{2}	53.2%	1.4×10^{2}	28.78%	9.2×10^{1}	32.7%	1.0×10^{2}	39.4%	1.2×10^{2}	42.8%
100	3.9×10^{2}	32.4%	3.7×10^{2}	47.08%	3.4×10^{2}	24.9%	3.0×10^{2}	26.5%	3.5×10^{2}	34.1%
320	1.4×10^{3}	45.0%	1.2×10^{3}	37.61%	1.1×10^{3}	36.2%	8.8×10^{2}	17.1%	1.1×10^{3}	39.8%
1.0×10^{3}	3.7×10^{3}	24.4%	3.9×10^{3}	9.21%	3.7×10^{3}	38.6%	2.8×10^{3}	6.8%	3.5×10^{3}	25.7%
3.2×10^{3}	1.2×10^{4}	27.8%	1.1×10^{4}	15.38%	9.9×10^{3}	26.6%	9.4×10^{3}	15.2%	1.1×10^{4}	23.3%
1.0×10^{4}	3.7×10^{4}	28.1%	3.4×10^{4}	13.76%	2.5×10^{4}	16.8%	2.8×10^{4}	9.7%	3.1×10^{4}	24.5%
1.0×10^{5}	3.7×10^{5}	33.6%	2.8×10^{5}	20.04%	2.5×10^{5}	20.1%	2.6×10^{5}	16.6%	2.9×10^{5}	29.4%
1.0×10^{6}	3.7×10^{6}	33.7%	3.0×10^{6}	19.94%	2.5×10^{6}	13.2%	2.9×10^{6}	16.3%	3.0×10^{6}	27.6%
1.0×10^{7}	3.7×10^{7}	22.5%	3.1×10^{7}	19.91%	2.6×10^{7}	10.6%	2.8×10^{7}	19.0%	3.0×10^{7}	22.7%

Table 15. B19 FDQA, v1 determination of range for donation testing: data analyzed with B19 QS1 through B19 QS5

Parvovirus B19 Input titer (IU/mL)	Mean Observed Parvovirus B19 titer (IU/mL)	Acceptable precision	Acceptable accuracy	In linear range
3.2	2.1×10^{1}	n	n	y
10	3.2×10^{1}	n	y	y
32	1.1×10^{2}	n	y	y
100	3.5×10^{2}	n	y	y
320	1.0×10^{3}	y	y	y
1.0×10^{3}	3.2×10^{3}	y	y	y
3.2×10^{3}	9.8×10^{3}	y	y	y
1.0×10^{4}	2.8×10^{4}	y	y	y
1.0×10^{5}	2.5×10^{5}	y	y	y

Range

Table 16. B19 FDQA, v1 determination of range for manufacturing pool testing: data analyzed with P1 through P8

Parvovirus B19 Input titer (IU/mL)	Mean Observed Parvovirus B19 titer (IU/mL)	Acceptable precision	Acceptable accuracy	In linear range
3.2	2.1×10^{1}	n	n	y
10	4.1×10^{1}	n	n	y
32	1.2×10^{2}	n	y	y
100	3.5×10^{2}	y	y	y
320	1.1×10^{3}	y	y	y
1.0×10^{3}	3.5×10^{3}	y	y	y
3.2×10^{3}	1.1×10^{4}	y	y	y
1.0×10^{4}	3.1×10^{4}	y	y	y
1.0×10^{5}	2.9×10^{5}	y	y	y
1.0×10^{6}	3.0×10^{6}	y	y	y
1.0×10^{7}	3.0×10^{7}	y	y	y

Range

Table 17. B19 FDQA, v1 precision results for donation testing: data analyzed with B19 QS1 through B19 QS5

Parvovirus B19 Input titer (IU/mL)	KGS		JJM		HV		Collective Data	
	Mean Obs. B19 titer (IU/mL)	% CV	Mean Obs. B19 titer (IU/mL)	% CV	Mean Obs. B19 titer (IU/mL)	% CV	Mean Obs. B19 titer (IU/mL)	% CV
1.0×10^3	3.7×10^3	16.0%	3.1×10^3	18.4%	3.6×10^3	11.3%	3.5×10^3	16.7%
1.0×10^5	3.4×10^5	12.4%	2.5×10^5	16.3%	3.2×10^5	15.0%	3.0×10^5	19.1%

Table 18. B19 FDQA, v1 precision results for manufacturing pool testing: data analyzed with P1 through P8

Parvovirus B19 Input titer (IU/mL)	KGS		JJM		JMP		Collective Data	
	Mean Obs. B19 titer (IU/mL)	% CV	Mean Obs. B19 titer (IU/mL)	% CV	Mean Obs. B19 titer (IU/mL)	% CV	Mean Obs. B19 titer (IU/mL)	% CV
1.0×10^3	4.0×10^3	15.8%	4.3×10^3	24.3%	6.2×10^3	9.3%	4.8×10^3	25.5%
1.0×10^5	3.4×10^5	12.2%	3.6×10^5	9.8%	5.6×10^5	7.5%	4.2×10^5	25.4%
1.0×10^7	3.0×10^7	12.7%	3.3×10^7	10.7%	4.9×10^7	3.9%	3.7×10^7	24.2%

Table 19. B19 FDQA, v1 validation results for robustness testing at threshold concentrations

Parvovirus B19 Input titer (IU/mL)	Number / Total B19 positive	% Positive	Mean Observed B19 titer (IU/mL)	Std. Dev.	% CV	IC C_T Average	IC C_T Range
NHP	0 / 20	0	N/A	N/A	N/A	34.94	33.51 to 37.23
2.0×10^3	20 / 20	100	6.3×10^3	1.7×10^3	27.8%	36.49	N/A
1.0×10^4	20 / 20	100	2.9×10^4	6.8×10^3	23.0%	37.89	N/A
5.0×10^4	20 / 20	100	1.4×10^5	3.2×10^4	22.7%	N/A	N/A
1.0×10^5	20 / 20	100	2.8×10^5	5.0×10^4	17.6%	N/A	N/A

Table 20. B19 FDQA, v1 validation results for robustness testing of reagent variability with MMX Lot 2

Parvovirus B19 Input titer (IU/mL)	Number / Total B19 positive	% Positive	Mean Observed B19 titer (IU/mL)	Std. Dev.	% CV	IC C_T Average	IC C_T Range
NHP	0 / 20	0	N/A	N/A	N/A	35.55	34.42 to 37.24
2.0×10^3	20 / 20	100	3.3×10^3	9.7×10^2	29.2%	36.33	N/A
1.0×10^4	20 / 20	100	1.7×10^4	3.4×10^3	19.5%	38.79	N/A
5.0×10^4	20 / 20	100	8.4×10^4	1.7×10^4	19.8%	39.71	N/A
1.0×10^5	20 / 20	100	1.7×10^5	4.0×10^4	23.0%	N/A	N/A

Table 21. B19 FDQA, v1 validation results for robustness testing of reagent variability with MMX Lot 3

Parvovirus B19 Input titer (IU/mL)	Number / Total B19 positive	% Positive	Mean Observed B19 titer (IU/mL)	Std. Dev.	% CV	IC C_T Average	IC C_T Range
NHP	0 / 20	0	N/A	N/A	N/A	34.99	34.08 to 36.65
2.0×10^3	20 / 20	100	4.4×10^3	9.9×10^2	22.5%	36.58	N/A
1.0×10^4	20 / 20	100	2.1×10^4	4.2×10^3	20.2%	38.50	N/A
5.0×10^4	20 / 20	100	1.1×10^5	2.6×10^4	24.3%	38.52	N/A
1.0×10^5	20 / 20	100	2.1×10^5	5.8×10^4	27.1%	N/A	N/A

Table 22. B19 FDQA, v1 validation results for robustness testing of cross-contamination: alternating B19 negative (NHP) and high-titer B19 positive (1.00 x 10^9 IU/mL B19) samples in a checkerboard pattern

Parvovirus B19 Input titer (IU/mL)	Number / Total B19 positive	% Reactive	B19 C_T Average	B19 C_T Range	Number / Total IC positive	IC C_T Average	IC C_T Range
NHP	0/15	0	N/A	N/A	15/15	35.52	34.46 to 37.45
1.00 x 10^9	15/15	100	14.19	13.94 to 14.55	N/A	N/A	N/A

11. Appendix A. Test Sample Preparation

Table A.1. Preparation of the Test Sample Panel for the Validation of Linearity, Accuracy, Limit of Detection, Limit of Quantitation, Range, and Robustness

Stock source	Volume of stock source	Diluent Source	Volume of diluent	Final sample and concentration	Final sample volume
2.7×10^{11} IU/mL Talecris In-house Quantitative Standard for the B19 FDQA, v1, NAT-126	185 µL	NAT-129	315 µL	1.0×10^{11} IU/mL Parvovirus B19 Intermediate	500 µL
1.0×10^{11} IU/mL Parvovirus B19 Intermediate	220 µL	NAT-129	21.78 mL	**1.0×10^{9} IU/mL Parvovirus B19 test panel NAT-127I**	22.00 mL
1.0×10^{9} IU/mL Parvovirus B19 Intermediate & test panel	360 µL	NAT-129	35.64 mL	**1.0×10^{7} IU/mL Parvovirus B19 test panel NAT-127E11**	36.00 mL
1.0×10^{7} IU/mL Parvovirus B19 Intermediate & test panel	3.60 mL	NAT-129	32.40 mL	**1.0×10^{5} IU/mL Parvovirus B19 test panel NAT-127E10**	36.00 mL
1.0×10^{6} IU/mL Parvovirus B19 Intermediate & test panel	3.80 mL	NAT-129	34.20 mL	**1.0×10^{5} IU/mL Parvovirus B19 test panel NAT-127E9**	38.00 mL
1.0×10^{5} IU/mL Parvovirus B19 Intermediate & test panel	4.80 mL	NAT-129	43.20 mL	**1.0×10^{4} IU/mL Parvovirus B19 test panel NAT-127E8**	48.00 mL
1.0×10^{4} IU/mL Parvovirus B19 Intermediate & test panel	15.36 mL	NAT-129	32.64 mL	**3.2×10^{3} IU/mL Parvovirus B19 test panel NAT-127E7**	48.00 mL
3.2×10^{3} IU/mL Parvovirus B19 Intermediate & test panel	15.00 mL	NAT-129	33.00 mL	**1.0×10^{3} IU/mL Parvovirus B19 test panel NAT-127E6**	48.00 mL
1.0×10^{3} IU/mL Parvovirus B19 Intermediate & test panel	15.36 mL	NAT-129	32.64 mL	**320 IU/mL Parvovirus B19 test panel NAT-127E5**	48.00 mL
320 IU/mL Parvovirus B19 Intermediate & test panel	15.00 mL	NAT-129	33.00 mL	**100 IU/mL Parvovirus B19 test panel NAT-127E4**	48.00 mL
100 IU/mL Parvovirus B19 Intermediate & test panel	15.36 mL	NAT-129	32.64 mL	**32 IU/mL Parvovirus B19 test panel NAT-127E3**	48.00 mL
32 IU/mL Parvovirus B19 Intermediate & test panel	13.75 mL	NAT-129	30.25 mL	**10 IU/mL Parvovirus B19 test panel NAT-127E2**	44.00 mL
10 IU/mL Parvovirus B19 Intermediate & test panel	10.56 mL	NAT-129	22.44 mL	**3.2 IU/mL Parvovirus B19 test panel NAT-127E1**	33.00 mL

- Prepare 24 x 1.00 mL aliquots of parvovirus B19 test panels NAT-127E1 - NAT-127E11, ranging from 32 to 1.0×10^{7} IU/mL for linearity, accuracy, limit of quantitation, limit of detection, and range studies.
- Prepare 15 x 1.00 mL aliquots of parvovirus B19 test panel NAT-127I at 1.0×10^{9} IU/mL for robustness study testing cross-contamination.

Table A.2. Preparation of the Test Sample Panel for the Validation of Precision: Repeatability and Intermediate Precision

Stock source	Volume of stock source	Diluent Source	Volume of diluent	Final sample and concentration	Final sample volume
1.0×10^9 IU/mL Parvovirus B19 Intermediate & test panel NAT-127I	350 µL	NAT-129	34.65	**1.0×10^7 IU/mL Parvovirus B19 test panel NAT-127F3**	35.00 mL
1.0×10^7 IU/mL Parvovirus B19 Intermediate & test panel	650 µL	NAT-129	64.35 mL	**1.0×10^5 IU/mL Parvovirus B19 test panel NAT-127F2***	65.00 mL
1.0×10^5 IU/mL Parvovirus B19 Intermediate & test panel	650 µL	NAT-129	64.35 mL	**1.0×10^3 IU/mL Parvovirus B19 test panel NAT-127F1***	65.00 mL

- Prepare 24 x 1.00 mL aliquots of parvovirus B19 test panels NAT-127F1 through NAT-127F3 at 1.0×10^3, 1.0×10^5 and 1.0×10^7 IU/mL B19 for the precision study.
  *NAT-127F1 and NAT-127F2 will also be utilized as diluent for specificity panels containing HBV.

Table A.3. Preparation of the Test Sample Panel for the Validation of Robustness at Threshold Concentrations

Stock source	Volume of stock source	Diluent Source	Volume of diluent	Final sample and concentration	Final sample volume
1.0×10^7 IU/mL Parvovirus B19 Intermediate & test panel NAT-127E11	460 µL	NAT-129	45.54 mL	**1.0×10^5 IU/mL Parvovirus B19 test panel NAT-127G4**	46.00 mL
1.0×10^5 IU/mL Parvovirus B19 Intermediate & test panel	17.50 mL	NAT-129	17.50 mL	**5.0×10^4 IU/mL Parvovirus B19 test panel NAT-127G3**	35.00 mL
5.0×10^4 IU/mL Parvovirus B19 Intermediate & test panel	6.80 mL	NAT-129	27.20 mL	**1.0×10^4 IU/mL Parvovirus B19 test panel NAT-127G2**	34.00 mL
1.0×10^4 IU/mL Parvovirus B19 Intermediate & test panel	5.60 mL	NAT-129	22.40 mL	**2.0×10^3 IU/mL Parvovirus B19 test panel NAT-127G1**	28.00 mL

- Prepare 20 x 1.00 mL aliquots of parvovirus B19 test panels NAT-127G1 through NAT-127G4 ranging from 2.0×10^3 to 1.0×10^5 IU/mL B19 for the robustness study at threshold concentrations.

Table A.4. Preparation of the Test Sample Panel for the Validation of Specificity

Stock source	Volume of stock source	Diluent Source	Volume of diluent	Final sample and concentration	Final sample volume
7.6×10^8 IU/mL HBV In-house standard	98 µL	NAT-129	1402 µL	5.0×10^7 IU/mL HBV Intermediate	1500 µL
5.0×10^7 IU/mL HBV Intermediate	320 µL	NAT-129	31.68 mL	**NHP + 5.0×10^5 IU/mL HBV Test panel NAT-127H1**	32.00 mL
5.0×10^7 IU/mL HBV Intermediate	320 µL	1.0×10^5 IU/mL Parvovirus B19 Intermediate NAT-127F2	32.00 mL	**1.0×10^5 IU/mL B19 + 5.0×10^5 IU/mL HBV Test panel NAT-127H3**	32.32 mL
5.0×10^7 IU/mL HBV Intermediate	320 µL	1.0×10^3 IU/mL Parvovirus B19 Intermediate NAT-127F1	32.00 mL	**1.0×10^3 IU/mL B19 + 5.0×10^5 IU/mL HBV Test panel NAT-127H2**	32.32 mL

- Prepare 24 x 1.00 mL aliquots of parvovirus B19 test panels NAT-127H1 through NAT-127H3 for specificity study in the presence of HBV.

12. Appendix B. Unplanned Deviations

The initial amplification of a test panel for robustness at threshold concentration by one operator gave invalid results due to an atypical amplification plot that suggested an AB7300 instrument error during amplification. A technical application specialist from Applied Biosystems was consulted to review the AB7300 run file from the invalid run. After reviewing the data file, the specialist concluded that the atypical amplification plot was due to an anomaly in the instrument that may have been caused by a brief power surge through the electrical supply. The robustness test panel was re-amplified on the same day using the same AB7300 instrument. The re-amplification of the original test panel nucleic acid extractions generated a normal amplification plot and the assay gave valid results according to the assay validity criteria. Subsequent B18 FDQA, v1 assay runs on this AB7300 instrument have also been valid.

One additional linearity test sample panel and precision test sample panel were prepared from the original 1.0×10^9 IU/mL B19 test sample intermediate (Table A.1) because insufficient volume of test sample at each concentration was available for repeat testing. One linearity test panel and two precision test panels were retested using the new test sample panels because the original extractions had exceeded their expiry. These linearity and precision test samples were amplified and analyzed with plasmid standards P1 through P8 for manufacturing pool testing. The repeat linearity and precision assays for manufacturing pool testing gave valid results according to the assay validity criteria.

NAT-129 was the NHP lot specified for this validation. For robustness testing of cross-contamination, insufficient volumes of NAT-129 were available. NHP from a different lot number, NAT-123, was used to prepare 2 NEG controls for this robustness assessment. NAT-123 is also a multiple-source plasma pool that tested negative for HIV-1, HCV, HBV, HAV, and parvovirus B19 using PCR methodologies.

13. Appendix C. Planned Deviations

None.

Plasma Donor

Test Procedure

Participation in Proficiency Studies

Number of Pages
(including cover page)

2

Original is signed

_____ _____

Name and Rank Date

_____ _____

Name and Rank Date

Valid from: 19 Jul 2007

Information on proficiency testing at the viral marker test laboratories is obtained through the organizations that supply Source Plasma to Talecris. The test laboratories participate in programs, in which a panel of reference samples is provided by an independent body (e.g., The College of American Pathologists (CAP), international Viral Quality Control program) to challenge the tests methods performed at the test laboratories against these reference samples.

The results as reported by the test laboratories are summarized in Table 1 below.

Table 1 - Summary of Proficiency Test Results at the Viral Marker Test Laboratories

Laboratory	Test (Method)	Test Panel	Frequency	Result
BioLife Testing Laboratory 2197 Parkway Lake Drive Hoover, Alabama 35244, USA	HBsAg (EIA 3.0; BioRad/PRISM; Abbott)	CAP	Quarterly	All acceptable
	Anti-HCV (ELISA 3.0, Ortho)	CAP	Quarterly	All acceptable
	Anti-HIV 1/2 Plus O (EIA; BioRad)	CAP	Quarterly	All acceptable
Life Sera, Inc. Testing Laboratory 780 Park North Blvd.; Ste 100 Clarkston, GA 30021, USA	HBsAg (Auszyme EIA; Abbott)	CAP	Quarterly	All acceptable
	Anti-HCV (EIA 2.0; Abbott)	CAP	Quarterly	All acceptable
	Anti-HIV 1/2 (rDNA EIA; Abbott)	CAP	Quarterly	All acceptable
South Texas Blood & Tissue Center 6211 IH 10 West San Antonio, Texas 78201, USA	HBsAg (PRISM; Abbott)	CAP	Quarterly	All acceptable
	Anti-HCV (EIA 2.0; Abbott)	CAP	Quarterly	All acceptable
	Anti-HIV 1/2 (rDNA EIA; Abbott)	CAP	Quarterly	All acceptable
ZLB Bioplasma, Inc. Test Laboratory 9320 Park West Bl. Knoxville, TN 37923, USA	HBsAg (Auszyme EIA; Abbott)	CAP	Quarterly	All acceptable
	Anti-HCV (EIA 2.0; Abbott)	CAP	Quarterly	All acceptable
	Anti-HIV 1/2 (rDNA EIA; Abbott)	CAP	Quarterly	All acceptable
Interstate Blood Bank, Inc. Test Laboratory 5700 Pleasant View Rd. Memphis, TN 38134, USA	HBsAg (Auszyme EIA; Abbott)	CAP	Quarterly	All acceptable
	Anti-HCV (EIA 2.0; Abbott)	CAP	Quarterly	All acceptable
	Anti-HIV 1/2 (rDNA EIA; Abbott)	CAP	Quarterly	All acceptable
Talecris Biotherapeutics, Inc. Raleigh Test Lab, 1200 New Hope Road, Raleigh, NC 27610, USA	HBV NAT (Roche COBAS)	CAP	Quarterly	All acceptable
	HCV NAT (Roche COBAS)	CAP	Quarterly	All acceptable
	HIV NAT (Roche COBAS)	CAP	Quarterly	All acceptable
National Genetics Institute 2440 S. Sepulveda Blvd., Suite 130 Los Angeles, CA 90064, USA	NGI UltraQual™ HBV Assay	CAP	Quarterly	All acceptable
	NGI UltraQual™ HCV Assay	CAP	Quarterly	All acceptable
	NGI UltraQual™ HIV Assay	CAP	Quarterly	All acceptable

Talecris Biotherapeutics, Inc.
Clayton, NC

T.18.22-08US

Plasma Donor

Plasma Collection Materials

Number of Pages
(including cover page)

4

Original is signed

_____ _____

Name and Rank Date

_____ _____

Name and Rank Date

Valid from: 23 Oct 2006

1. Plasma Collection Materials

1.1 Description of Automated Plasmapheresis

Plasma is collected using a single venipuncture and closed-system separating device at the donor bedside. All fluid path equipment used in the process is sterile and for single use only. The donor blood flows under venous pressure into the needle and tubing and is mixed with anticoagulant before entering the separating device. There are three types of devices in common use, employing either a flow through centrifuge bowl or using a rotating filter. All devices are computer controlled. The machines go through several cycles that collect whole blood and separate the plasma from the cells, which are returned to the donor. The amount of plasma collected is determined from established nomograms based on donor physical parameters. The devices are programmed to continue collecting plasma until a preset amount is obtained. The preset amount of plasma is determined by the weight of plasma on an electronic scale that is part of the bedside device. Automated plasma collection may be performed with or without the use of saline fluid replacement. Since this is an individual donor process, the risk of returning cells to the wrong donor is nonexistent.

1.2 Plasma Collection Containers and Anticoagulant Solution

A listing of the plasma collection containers, anticoagulants used and the specifications for each are provided in the attached tables. High Density Polyethylene (HDPE) Plasma Collection Bottles and 4% Sodium Citrate Anticoagulant solution are used in the collection process for plasma supplied to Talecris.

Table 1 - Haemonetics Corporation Plasma Pooling Bottle

Name of Product	Manufacturer	Specification		Licensing/Registration	
		Test	Spec	Competent Authority	Type of Licensing/Registration
Plasma Pooling Bottle Product codes: 694, 694S, 694D	Haemonetics Leetsdale Facility, Building 18 Avenue C, Leetsdale, PA, USA, 15058	Identity	HDPE	FDA	510(k) BK970029, BK910009 and BK020001
		Leak Test for Seal Integrity	Pass		
		Visual Inspection	Pass		
		Sterility (Gamma Radiation)	SAL 10^{-6}		

Table 2 - Baxter Healthcare Corporation Plasma Pooling Bottle

Name of Product	Manufacturer	Specification		Licensing/Registration	
		Test	Spec	Competent Authority	Type of Licensing/Registration
Plasma Pooling Bottle Product code: 4R2065, 4R2067, 4R2068, 4R2069	Baxter Healthcare Corp 911 N. Davis Street Cleveland, MS, USA, 38732	Identity	HDPE PL1539	FDA	510(k) BK830009
		Leak Test for Seal Integrity	Pass		
		Visual Inspection	Pass		
		US Physico-Chemical Tests for Polymers (USP <661>)	Pass		
		Sterility (Gamma Radiation)	SAL 10^{-6}		
		Pyrogenicity	<20 EU/device		

Table 3 - Haemonetics Corporation 4% Sodium Citrate Anticoagulant Solution

Name of Solution	Manufacturer	Anti-coagulant Solution	Specification				Licensing/Registration	
			Bag		Solution		Competent Authority	Type of Licensing/ Registration
			Test	Spec	Test	Spec		
Anti-coagulant Sodium Citrate Solution, USP Product Code: 420A	Haemonetics Corporation 155 Medical Sciences Dr. Union, SC, USA, 29379	4% Sodium Citrate	Polyvinyl Chloride homopolymer	100[a]	Visual	Clear to Amber	US FDA	NDA 98-0123
			Nonvolatile Residue	NMT 0.015 g	Assay sodium citrate	3.80 to 4.20%		
			Buffering Capacity	Pass	pH	6.4 to 7.5		
			Heavy Metals	Pass	Sterility	Pass		
			Microbial Count	NMT 100 CFU	Bacterial Endotoxins	Pass		

[a] Parts by weight.

Table 4 - Baxter Healthcare Corporation 4% Sodium Citrate Anticoagulant Solution

Name of Solution	Manufacturer	Anti-coagulant Solution	Specification				Licensing/Registration	
			Bag		Solution		Competent Authority	Type of Licensing/ Registration
			Test	Spec	Test	Spec		
Anti-coagulant Sodium Citrate Solution, USP Product Codes: 4B7867Q and 4B7889Q	Baxter Healthcare Corporation, Highway 221 N., Marion, NC, USA, 28752	4% Sodium Citrate	Polyvinyl Chloride resin	56 to 66%	Color, Filter until clear per USP	Clear	FDA	NDA 77-923
			Di-ethyl-hexyl-phthalate (DEHP)	24 to 30%	Sodium citrate dihydrate	38.0 to 42.0 g/L		
			Epoxidized linseed oil	7 to 11%	pH	6.4 to 7.5		
			CZ-11 powder	0.06 to 0.2%	Sterility	Pass		
			Acrawax C	0.1 to 0.5%	Pyrogen testing per USP	Pass		

Talecris Biotherapeutics, Inc.
Clayton, NC

T.18.25-03US

Plasma Donor

Freezing, Storage and Transport of Source Plasma

Number of Pages
(including cover page)

2

Original is signed

_____ _____

Name and Rank Date

_____ _____

Name and Rank Date

Valid From: 10 Jan 2005

All Source Plasma is frozen and stored at -20°C or colder in accordance with the U.S. Code of Federal Regulations, 21CFR640, Subpart G. Source Plasma for further manufacture by Talecris will expire three years after the date of collection.

Frozen plasma is stored and transported in conditions designed to maintain the temperature at or below -20°C. The temperature is measured by means of a probe immersed in glycerol/water or suitable alternate to simulate the temperature of the plasma. Minimum and maximum load validation studies were performed to verify that transport trucks are capable of maintaining plasma temperatures of -20°C or colder. Routine operations such as defrost cycles and loading/unloading of the plasma may cause the temperature to exceed -20°C. These routine occurrences are tracked and documented to ensure compliance with the US CFR storage and transport requirements.

Transport Flow from Plasma Suppliers to the Fractionation Site

Plasma is collected, frozen and stored under the control of the plasma collection organizations until all test results are received and the HBV, HCV and HIV reactive/positive plasma units are removed. As necessary, plasma units may be transported to an interim off-site storage facility. Non-reactive/negative plasma units are transported to the fractionation site by approved establishments inspected and deemed satisfactory by Talecris Quality Operations.

Product: Plasma

Plasma Inventory Hold Procedure

Number of Pages
(including cover page)

2

Original is signed

_____ _____

Name and Rank Date

_____ _____

Name and Rank Date

Valid from: 22 Jun 2004

A quality assurance system is in place to verify that every plasma unit for each individual manufacturing pool has been held for a minimum of 60 days. This time period is measured from the date of plasma unit donation and is consistent with the requirement of the PPTA 60-day Inventory Hold Voluntary Standard. The inventory hold period allows the manufacturing site to perform look-back investigation(s) for any notifications received from suppliers of plasma regarding a change in plasma donor qualification status. If a plasma donor tests positive for a viral marker or new information is received that causes a donor to no longer be acceptable for plasma donation at any time, all unprocessed negative plasma units collected from that donor are traced and destroyed prior to pooling for fractionation.

Plasma donors are not specifically retested for markers of infection at the end of the 60-day inventory hold period. However, only plasma units collected from donors who have completed two batteries of plasma donation screening interviews and laboratory tests are acceptable for further manufacture into plasma-derivatives. This procedure is in accordance with the PPTA Qualified Donor Voluntary Standard. The second battery of plasma donation screening interviews and laboratory tests must be performed prior to receiving any plasma units from a donor. If the second qualifying plasma donation screening interview and laboratory testing is not performed then the initial plasma donation is unacceptable for the manufacture of plasma-derivatives.

Talecris Biotherapeutics, Inc.
Clayton, NC

T.18.08-01

Plasma Pool

Manufacturing Site

Name: Talecris Biotherapeutics, Inc.
Address: 8368 US 70 West
Clayton, North Carolina, USA
27520

Original is signed

Name and Rank Date

Name and Rank Date

Valid from: 19 May 2005

Talecris Biotherapeutics, Inc.
Clayton, NC

T.18.07-05US

Plasma Pool

Specification and Test Procedure for In-Process Controls

Number of Pages
(including cover page)

3

Original is signed

Name and Rank Date

Name and Rank Date

Valid from: 18 Oct 2006

1. Criteria for Acceptance/Rejection of Plasma Units

Prior to processing plasma units for pooling, the bar coded unique identifying number for every plasma unit is 100% checked and verified against submitted test results and purchase specifications. This process also verifies lookback and post donation information received from each plasma supplier. Further control of the plasma units is assured by 100% visual inspection. Each and every plasma unit is verified to be acceptable for further manufacture.

Units in the following categories are rejected:

- Hemolyzed (red) units;
- Unidentified/extra units having no available documentation;
- Thawed units;
- Broken units;
- Units with missing labels;
- Units identified by the PUV system as being unacceptable.

The 100% PUV scanning and visual inspection is followed by a Quality Operations (QO) audit of each modeled plasma manufacturing pool.

2. Characterization of the Plasma Manufacturing Pool

The plasma pools manufactured at the Clayton, NC, USA plasma fractionation facility are typically made up of 3700 to 3900 liters of starting material (Source Plasma for further manufacture), corresponding to approximately 4500 to 4750 single donations of Source Plasma. Only plasma from qualified donors defined by the PPTA Qualified Donor Voluntary Standard and which meets the viral test specifications is acceptable for further manufacture. The plasma is eligible for processing if 60 days time has elapsed from the date of collection and the plasma is aged not more than (NMT) 3 years from the date of collection. The plasma pool is the same for all products and is fractionated by the Cohn-Oncley plasma fractionation process.

3. Sampling of the Plasma Pool and Storage of the Samples

The plasma is thawed until a homogeneous pool solution is achieved. Qualified coordinators collect sample vials from every plasma pool for microbiological testing, viral testing and reserve samples. Reconciling the pool number against the schedule provides assurance that samples from each pool are stored. Records and samples of each pool are stored for not less than (NLT) 1 year after the expiration date of the finished product with the longest shelf life.

4. Specification and In-Process Controls

A second round of viral marker and NAT testing is performed for each plasma manufacturing pool. All plasma manufacturing pools must meet the specifications which are listed in the document titled *Test Specifications for the Plasma Manufacturing Pool* in order to be acceptable for manufacture into plasma derivatives.

The following in-process controls apply to each plasma manufacturing pool:

Test	Requirement	Method
Temperature	5°C or colder	Continuous Monitor Probe
Bioburden	Action Level: 100 cfu/mL[a]	Plate Count

[a] An action level excursion results in an investigation by the Manufacturing and Quality units. An assessment of product safety is performed based on the results of the investigation in combination with all other testing to determine final product release status.

Talecris Biotherapeutics, Inc.
Clayton, NC

T.18.51-02

Plasma Donor

System in Place Between Manufacturer and Plasma Supplier

Number of Pages
(including cover page)

3

Original is signed

_____ _____

Name and Rank Date

_____ _____

Name and Rank Date

Valid from: 12 Jul 2007

All approved plasma collection organizations as indicated in Chapter 2.1.1.a. have signed a contract whose terms include specific quality criteria, e.g., Source Plasma Specifications (collection materials, plasma donor criteria, plasma donation specifications), storage and transport requirements, donation tracing, and communication of post donation information.

A quality assurance system is required to be established, documented and maintained for each plasma collection organization. The Quality Assurance program in place for suppliers of plasma includes:

- Preparation of Standard Operating Procedures
- Establishment of records so that donations can be traced including a unique donation identifying number, date of collection, quality control testing performed and results
- Control of labeling, storage and transport of donations
- Establishment of quality audits/review
- Adherence to specifications for Source Plasma for further manufacture into medicinal products

The established systems for notification between the manufacturer and the plasma collection organizations are in compliance with Directive 2002/98/EC, amending Directive 2001/83/EC and Commission Directive 2005/61/EC as well as the US CFR and FDA regulations.

Verification of Contract Application in all Centers

Adherence to the quality specifications as stated in the respective contracts is verified by Quality Operations through performing regular audits of the collection centers and test facilities. Audits are performed by Quality Operations personnel at new locations for collection or test sites before the first plasma donations are supplied, and periodically thereafter based on compliance performance.

For each audit, a report is issued. The report includes any deviations observed or any improvements to be implemented. Should a serious failure be discovered at a plasmapheresis center, acceptance of further plasma shipments from that center would be rejected and a possible impact of that failure on previously shipped plasma would be considered. In accordance with the Specification for Source Plasma the plasma collection organizations are required to notify the manufacturer immediately if any serious failure at a center should occur. Finally, at the end of each calendar year an overview of all audits performed is evaluated by Quality Operations.

These established procedures verify the fulfillment of the GMP/Good Practice requirements in accordance with GMP Directive 2003/94/EC (including Annex 14 of the EU Guide to GMP) and Commission Directive 2005/62/EC and other relevant GMP requirements at each establishment.

Manufacturer's Commitments

Contents of the standard contract with each plasma collection organization is confirmed by a responsible person on a yearly basis. In the event of a serious failure of a plasmapheresis center being discovered, the FDA, the European Medicines Agency (EMEA) and/or other relevant national authorities will be informed.

Contracts between the Manufacturer and Third Parties

A standard contract is in place between the manufacturer and any third party, which plasma derived products or intermediates are supplied to, defining the roles and responsibilities with regards to GMP requirements and notification.

SPECIFICATION – RAW MATERIAL

Material	**Sodium Caprylate (Sodium Octanoate)**				
Item Code	1000007008			**Department**	**Quality Control**
Specification No.	RLS2/QC/RM/SPC015	**Revision**	01	**Effective Date**	11 Jan 08
Supersedes	00	**Dated**	12 Jun 07	**Page No.**	Page 1 of 3
Next Review	Two years from Effective Date				

Storage conditions	In a dry place at room temperature in well-closed containers.	USP
Re-testing period	One year.	
Sample quantity	30g + (Retention sample : 2 × 30g)	
Critical Raw material	Yes	

S. No.	Test	Specification	Ref
01	Characters	A white, crystalline powder, very soluble or freely soluble in water, freely soluble in acetic acid, sparingly soluble in alcohol, practically insoluble in acetone.	BP
02	Identification	A. The retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with reference solution. B. Gives the reaction of sodium.	BP
		A. The retention time of the major peak in the chromatogram of Test solution 1 corresponds to that in the chromatogram of the Reference solution, as obtained in the test for Chromatographic purity. B. Meets the requirements.	USP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION – RAW MATERIAL					
Material	**Sodium Caprylate (Sodium Octanoate)**				
Item Code	1000007008			**Department**	**Quality Control**
Specification No.	RLS2/QC/RM/SPC015	**Revision**	01	**Effective Date**	11 Jan 08
Supersedes	00	**Dated**	12 Jun 07	**Page No.**	Page 2 of 3
Next Review	Two years from Effective Date				

S. No.	Test	Specification		Ref
03	Appearance of solution	Solution is clear and colourless.		BP/USP
04	pH	8.0 to 10.5.		BP
		between 8.0 and 10.5		USP
05	Related substances	Content of any related substance is not greater than 0.3 per cent. The sum of the related substances is not greater than 0.5 percent.		BP
06	Chromatographic Purity	Any impurity Total impurities	: Not more than 0.3% : Not greater than 0.5%	USP
07	Heavy metals	Complies with limit test for heavy metals (10 ppm).		BP
		Limit is 5 µg per g.		USP
08	Water	Not more than 3.0 per cent.		BP
		Not more than 3.0%.		USP
09	Assay (Anhydrous substance)	Not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of sodium octanoate.		BP/USP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION – RAW MATERIAL					
Material	**Sodium Caprylate (Sodium Octanoate)**				
Item Code	1000007008			**Department**	**Quality Control**
Specification No.	RLS2/QC/RM/SPC015	**Revision**	01	**Effective Date**	11 Jan 08
Supersedes	00	**Dated**	12 Jun 07	**Page No.**	Page 3 of 3
Next Review	Two years from Effective Date				

Parameters for re-test:

S. No.	Test	Specification	Ref
01	Characters	A white, crystalline powder, very soluble or freely soluble in water, freely soluble in acetic acid, sparingly soluble in alcohol, practically insoluble in acetone.	BP
02	Water	Not more than 3.0 per cent.	BP
		Not more than 3.0%.	USP
03	Assay (Anhydrous substance)	Not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of sodium octanoate.	BP/USP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

STANDARD TESTING PROCEDURE – RAW MATERIAL					
Material	**Sodium Caprylate (Sodium Octanoate)**				
Item Code	1000007008			**Department**	**Quality Control**
STP No.	RLS2/QC/RM/STP015	**Revision**	00	**Effective Date**	Jun 12, '07
Supersedes	None	**Dated**	NA	**Page No.**	Page 1 of 9
Next Review on	Maximum of 2 years from effective date				

01	Characters	A white, crystalline powder, very soluble or freely soluble in water, freely soluble in acetic acid, sparingly soluble in alcohol, practically insoluble in acetone.	BP

Apparatus and Chemicals:

100 ml Measuring cylinder

Clean Petri dish

Plain white paper

Clean glass test tubes

Water

Acetic acid

Alcohol

Acetone

Procedure:

1) Take 5g of sample in a clean and dry Petri dish. Place a plain white paper in a well-cleaned area and place the Petri dish on it, record observations about the following descriptive properties.
   a) Color (White, yellow, etc.)
   b) Physical state (Crystalline, amorphous, etc.)
2) Solubility:

   Take
   a) 1 g of sample and add 10 ml of water (Freely soluble).
   b) 1 g of sample and add 10 ml of acetic acid (Freely soluble)
   c) 0.1 g of sample and add 100 ml of alcohol (Sparingly soluble)

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

d) 0.01 g of sample and add 100 ml of acetone (Practically insoluble)

02	Identification	A. The retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with reference solution. B. Gives the reaction of sodium.	BP

A.

Apparatus and Chemicals:

As per test No. 05

Procedure:

Examine the chromatograms obtained in the Test No. 05 of the related substances.

The retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with reference solution (a).

B.

Apparatus and chemicals:

Analytical balance

10ml measuring cylinder

Water

Potassium carbonate

Potassium antimonite

Ice

Water bath

Procedure:

Solution S:

Dissolve 2.5 g of substance to be examined in carbon dioxide-free water and dilute to 25 ml with the same solvent.

Take 0.5 ml of Solution S, add 1.5 ml of methoxyphenylacetic reagent and cool in ice-water for 30 min. A voluminous, white, crystalline precipitate is formed. Place in water at 20 °C and stir for 5 min. The precipitate does not disappear. Add 1 ml of dilute ammonia. The precipitate dissolves completely. Add 1 ml of ammonium carbonate solution. No precipitate is formed.

03	Appearance of solution	Test solution is clear and colourless.	BP

Apparatus and Chemicals:

2 identical tubes of colourless, transparent, neutral glass with a flat base and internal diameter of 15 to 25 mm

Water

Procedure:

Dilute 20 ml of sample to 100 ml with water (Solution S). Compare the appearance of Solution S using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm compare a 40-mm layer of the liquid being examined with a 40-mm layer of water. Examine the columns of liquid in diffused daylight by viewing down the vertical axes of the tubes against a white background. The liquid is clear if its clarity is the same as that of water.

04	pH	8.0 to 10.5.	BP

Apparatus and Chemicals:

pH meter

100 ml beaker

Carbon dioxide-free water

Analytical balance

Procedure:

Take 1.0 g of sample and add 30 ml of carbon dioxide-free water. Immerse the electrodes in the solution and measure the pH at the same temperature as for the standard solutions. At the end of a set of measurements, record the pH of the solution used to standardize the meter and electrodes. Repeat the measurements, if the difference between this reading and the original value is greater than 0.05.

05	Related substances	Content of any related substance is not greater than 0.3 per cent. The sum of the related substances is not greater than 0.5 percent.	BP

Apparatus:

Gas chromatograph with head-space sampler

Water

Sulphuric acid

Ethyl acetate

Anhydrous sodium sulphate

Caprylic acid CRS

Procedure:

Test solution: Dissolve 0.116 g in water and dilute to 5 ml with the same solvent. Add 1 ml of a 2.8 per cent V/V solution of sulphuric acid and shake with 10 ml of ethyl acetate. Separate the organic layer and dry over anhydrous sodium sulphate.

Reference solution (a): Dissolve 0.10 g of caprylic acid CRS in ethyl acetate and dilute to 10 ml with the same solvent.

Reference solution (b): Dilute 1 ml of the test solution to 100 ml with ethyl acetate. Dilute 5 ml of the solution to 50 ml with ethyl acetate.

Gas chromatography conditions:

Column	:	fused silica column
Length	:	30 m
Diameter	:	0.25mm

Film thickness	:	0.25µm
Stationary phase	:	macrogol 20 000 2-nitroterephthalate
Carrier gas	:	helium for chromatography
Flow rate	:	1.5 ml/min
Detector	:	flame-ionisation detector
Split ratio	:	1:100

Temperature programme:

	Time (min)	Temperature (°C)	Rate (°C/min	Comment
Column	0-1 1-25 25-35	100 100 → 220 220	5	Isothermal Linear gradient isothermal
Injection port		250		
Detector		250		

Inject 1 µl of reference solution (b). The test is not valid unless in the chromatogram obtained the principal peak has a signal-to-noise ratio is at least 5.

Inject 1 µl of the test solution and 1 µl of reference solution (a). Calculate the percentage of related substances from the areas of the peaks in the chromatogram obtained with the test solution by the normalization procedure, disregarding any peaks with an area

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) and any peak due to the solvent. The substances is not greater than 0.5 per cent.

06	Heavy metals	Complies with limit test for heavy metals (10 ppm).	BP

Apparatus and chemicals:

Lead standard solution

Nessler Cylinder

Procedure:

Dissolve 2.0 g of substance to be examined in glacial acetic acid and dilute to 10 ml with the same acid. Add 10 ml of alcohol. Dissolve 12 ml of the solution in an organic solvent containing a minimum percentage of water, such as 1,4-dioxan or acetone containing 15% v/v of water. Take 12 ml of the solution, add 2 ml of acetate buffer pH 3.5, mix, add 1.2 ml of thioacetamide reagent, mix immediately and allow to stand for 2 minutes. Any brown colour produced is not more intense than that obtained by treating in the same manner a mixture of 10 ml of lead standard solution (1 ppm Pb), and 2 ml of the solution being examined. The standard solution exhibits a slightly brown colour when compared to a solution prepated by treating in the same manner a mixture of 10 ml of water and 2 ml of the solution being examined.

Preparation of Lead standard solution:

Take 0.4 g of lead nitrate, dilute to 250 ml with water (to produce 0.1% Pb). Dilute volume of the solution to 100 volumes with water (to produce 10 ppm Pb). Dilut

volume of the 10 ppm Pb solution to 10 volumes with water immediately before use to get 1 ppm Pb solution.

07	Water	Not more than 3.0 per cent.	BP

Apparatus and Chemicals:

KF Apparatus

KF Reagent

Methanol

Procedure:

Take about 20 ml of methanol into the titration vessel and titrate to the electrometric end point with the Karl Fischer reagent. Transfer quickly 2.0 g of sample being examined, accurately weighed to the titration vessel. Stir for 1 minute and titrate again to the electrometric end point using the Karl Fischer reagent.

The water content of the sample is given by the expression,

$$\text{Water content} = \frac{\text{Titer value x KF factor x 100}}{\text{Weight of the sample taken (in g)}}$$

$$= \quad \%$$

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

08	Assay (Anhydrous substance)	Not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of sodium octanoate.	BP

Apparatus and chemicals:

Burette

Anhydrous acetic acid

0.1 M perchloric acid

Procedure:

Dissolve 0.150 g of substance to be examined in 50 ml of anhydrous acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically

Calculate the purity of sodium caprylate by using the following formula.

$$\text{Assay} = \frac{\text{Titer value x Molarity of perchloric acid X 16.62 X 100}}{\text{0.1 x Sample taken in mg}}$$

= %

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

ANALYTICAL TEST REPORT

Product Information

Material : Sodium Caprylate (Sodium Octanoate)
Item Code : 1000007008
Batch No. :
Analysis Status :
Specification No. : RLS2/QC/RM/SPC015-01
GRN No. :
ATR No. : RLS2/QC/RM/ATR015-01
AR No. :

Sampling Information

Collected By :
Receipt Date :

Analysis Information

S. No.	Parameters	Result	Specification	Ref
01).	Characters		A white, crystalline powder, very soluble or freely soluble in water, freely soluble in acetic acid, sparingly soluble in alcohol, practically insoluble in acetone.	BP
02).	Identification		A. The retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with reference solution.	BP
			B. gives the reaction of sodium.	BP
			A. The retention time of the major peak in the chromatogram of Test solution 1 corresponds to that in the chromatogram of the Reference solution, as obtained in the test for Chromatographic purity.	USP
			B. Meets the requirements.	USP
03).	Appearance of solution		Solution is clear and colourless.	BP / USP
04).	pH		8.0 to 10.5.	BP
			between 8.0 and 10.5	USP
05).	Related substances		Content of any related substance is not greater than 0.3 per cent.	BP
			The sum of the related substances is not greater than 0.5 percent.	

Result : Sample meets / Does not meets the requirements as per specification.

Analysed By :
Date :
Reviewed By :
Date :

Quality Control

ANALYTICAL TEST REPORT

Product Information

Material : Sodium Caprylate (Sodium Octanoate)
Item Code : 1000007008
Batch No. :
Analysis Status :
Specification No. : RLS2/QC/RM/SPC015-01
GRN No. :
ATR No. : RLS2/QC/RM/ATR015-01
AR No. :

Sampling Information

Collected By :
Receipt Date :

Analysis Information

S. No.	Parameters	Result	Specification	Ref
06).	Chromatographic Purity			USP
	Any impurity		Not more than 0.3%	
	Total impurities		Not greater than 0.5%	
07).	Heavy metals		Complies with limit test for heavy metals (10 ppm).	BP
			Limit is 5 µg per g.	USP
08).	Water		Not more than 3.0 per cent.	BP
			Not more than 3.0%.	USP
09).	Assay (Anhydrous substance)		Not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of sodium octanoate.	BP / USP

Result : Sample meets / Does not meets the requirements as per specification.

Analysed By :
Date :

Reviewed By :
Date :

Quality Control

ANALYTICAL TEST REPORT

Product Information

Material : Sodium Caprylate (Sodium Octanoate) | Item Code : 1000007008
Batch No. : VP2727781 | Analysis Status : Certified
Specification No. : RLS2/QC/RM/SPC015-01 | GRN No. : 500428731
ATR No. : RLS2/QC/RM/ATR015-01 | AR No. : AR/471/01/3926

Sampling Information

Collected By : | Receipt Date : 25.01.09

Analysis Information

S. No.	Parameters	Result	Specification	Ref
01).	Characters	white crystalline powd. solubility complies	A white, crystalline powder, very soluble or freely soluble in water, freely soluble in acetic acid, sparingly soluble in alcohol, practically insoluble in acetone.	BP
02).	Identification	complies	A. The retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with reference solution.	BP
		complies	B. gives the reaction of sodium.	BP
		complies	A. The retention time of the major peak in the chromatogram of Test solution 1 corresponds to that in the chromatogram of the Reference solution, as obtained in the test for Chromatographic purity.	USP
		complies	B. Meets the requirements.	USP
03).	Appearance of solution	clear and colourless	Solution is clear and colourless.	BP / USP
04).	pH	9.5	8.0 to 10.5.	BP
		9.5	between 8.0 and 10.5	USP
05).	Related substances	complies	Content of any related substance is not greater than 0.3 per cent.	BP
		complies	The sum of the related substances is not greater than 0.5 percent.	BP

Result : Sample meets / ~~Does not meets~~ the requirements as per specification.

Analysed By : | Reviewed By :
Date : | Date :

Control

ANALYTICAL TEST REPORT

Product Information

Material :
Batch No. :
Specification No. :
ATR No. :

Item Code :
Analysis Status :
GRN No. :
AR No. :

Sampling Information

Collected By :

Receipt Date : 25.01.09

Analysis Information

S. No.	Parameters	Result	Specification	Ref
06).	Chromatographic Purity			
	Any impurity	Complies	Not more than 0.3%	USP
	Total impurities	Complies	Not greater than 0.5%	
07).	Heavy metals	Complies	Complies with limit test for heavy metals (10 ppm).	BP
		Complies	Limit is 5 µg per g.	USP
08).	Water	1.7 %	Not more than 3.0 per cent.	BP
		1.7 %	Not more than 3.0%.	USP
09).	Assay (Anhydrous substance)	99.7 %	Not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of sodium octanoate.	BP / USP

Result : Sample meets / ~~Does not meets~~ the requirements as per specification.

Analysed By :
Date :

Reviewed By :
Date :

Certificate of Analysis

http://certificates.merck.de

Date of print: 30.01.2008

8.17081.9010 Sodium caprylate PhEur

Batch VP897981

	Spec. Values		Batch Values	
Appearance	Almost white fine cristal powder		passes test	
Appearance of solution (10 %; water)	Clear and colorless to almost colorless		passes test	
Assay (Perchloric acid titration, calc. on anhydrous substance)	99.0 - 101.0	%	100.3	%
Identity	passes test		passes test	
Related substances (GC)				
largest single impurity	≤ 0.3	%	≤ 0.3	%
sum of all impurities	≤ 0.5	%	≤ 0.5	%
Water (according to Karl Fischer)	≤ 3.0	%	1.3	%
Heavy metals (as Pb)	≤ 0.001	%	≤ 0.001	%
pH-value (10 %; water)	8.0 - 10.5		9.5	
Residual solvents (Ph. Eur./ICH)	excluded by production process		passes test	
Endotoxins	≤ 20	U/g	≤ 20	U/g

Date of examination (DD.MM.YYYY): *16.10.2007*
Minimum shelf life (DD.MM.YYYY): *31.10.2012*

Corresponds Pha Eur

This document has been produced electronically and is valid without a signat

SPECIFICATION – RAW MATERIAL					
Material	**Sodium chloride**				
Item Code	**1000001486**			**Department**	**Quality Control**
Specification No.	RLS2/QC/RM/SPC016	**Revision**	00	**Effective Date**	Jun 19, '07
Supersedes	None	**Dated**	NA	**Page No.**	Page 1 of 4
Next Review	Two years from Effective Date				

Storage conditions	Store in tightly-closed containers.	IP
Re-test date	One year	
Sample quantity	30 gm + (2 × 30 gm)	

S. No.	Test	Specification	Ref
01	Characters	A white, crystalline powder or colourless crystals or white pearls, freely soluble in water, practically insoluble in ethanol.	BP
	Description	Colourless crystals or white, crystalline powder.	IP
02	Solubility	Freely soluble in water and slightly more soluble in boiling water, practically insoluble in ethanol.	IP
03	Identification	A. It gives the reaction of chlorides. B. It gives the reaction of sodium.	BP
		A. Gives the reaction of chlorides. B. Gives the reaction of sodium salts.	IP
04	Appearance of solution	Clear and colourless	BP
	Clarity and colour of solution	Clear and colourless	IP
05	Acidity or alkalinity	Not more than 0.5ml of 0.01M hydrochloric acid or of 0.01M sodium hydroxide is required.	BP
		Not more than 0.5ml of 0.01M hydrochloric acid or of 0.01M sodium hydroxide is required.	IP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION – RAW MATERIAL					
Material	Sodium chloride				
Item Code	1000001486			**Department**	Quality Control
Specification No.	RLS2/QC/RM/SPC016	**Revision**	00	**Effective Date**	Jun 19, '07
Supersedes	None	**Dated**	NA	**Page No.**	Page 2 of 4
Next Review	Two years from Effective Date				

S. No.	Test	Specification	Ref
06	Arsenic	Complies with the limit test for arsenic(1ppm)	IP/BP
07	Magnesium and alkaline-earth metals	Not more than 100 ppm (calculated as Ca)	BP
	Calcium and Magnesium	Not more than 50 ppm.	IP
08	Iron	Complies with the limit test for iron (2ppm)	BP
		Complies with the limit test for iron (20ppm)	IP
09	Barium	Opalescence in the solution is not more than that in a mixture of 5 ml of solution and distilled water.	BP
		No turbidity is produced within 2 hours.	IP
10	Bromide	The absorbance of the solution is not greater than that of standard (50 ppm).	BP
		Any violet colour produced is not more intense than that of standard.	IP
11	Heavy metals	Complies with limit test for heavy metals (5 ppm).	BP
		Not more than 5ppm	IP
12	Ferrocyanide	No blue colour develops within 10 min.	BP
		No blue colour is produced within 10 minutes	

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION – RAW MATERIAL					
Material	**Sodium chloride**				
Item Code	**1000001486**			**Department**	**Quality Control**
Specification No.	RLS2/QC/RM/SPC016	**Revision**	00	**Effective Date**	Jun 19,'07
Supersedes	None	**Dated**	NA	**Page No.**	Page 3 of 4
Next Review	Two years from Effective Date				

S. No.	Test	Specification	Ref
13	Iodide	The substance shows no blue colour.	BP
		The substance shows no blue colour after 5 minutes.	IP
14	Loss on drying	Not more than 0.5 per cent.	BP
		Not more than 1.0%.	IP
15	Phosphates	Complies with the limit test for phosphates (25 ppm).	BP
16	Sulphates	Complies with the limit test for sulphates (200ppm)	BP
		Complies with the limit test for sulphates (300ppm)	IP
17.	Aluminium	Not more than 0.2 ppm.	IP/BP
18.	Nitrites	The absorbance is not greater than 0.01.	BP
19.	Potassium (flame photometry)	Contains not more than 500 ppm of K	BP
		Not more than 0.1%	IP
20	Bacterial endotoxins	Less than 5 IU/g.	BP
21	Assay (Dried Substance)	Not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of NaCl	BP
		Not less than 99.0 per cent and not more than 100.5 per cent of NaCl.	IP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION – RAW MATERIAL					
Material	**Sodium chloride**				
Item Code	**1000001486**			**Department**	**Quality Control**
Specification No.	RLS2/QC/RM/SPC016	**Revision**	00	**Effective Date**	Jun 19, '07
Supersedes	None	**Dated**	NA	**Page No.**	Page 4 of 4
Next Review	Two years from Effective Date				

Parameters for re-test:

S. No.	Test	Specification	Ref
01	Characters	A white, crystalline powder or colourless crystals or white pearls, freely soluble in water, practically insoluble in ethanol.	BP
	Description	Colourless crystals or white, crystalline powder.	IP
02	Appearance of solution	Clear and colourless	BP
	Clarity and colour of solution	Clear and colourless	IP
03	Assay (Dried Substance)	Not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of NaCl	BP
		Not less than 99.0 per cent and not more than 100.5 per cent of NaCl.	IP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

STANDARD TESTING PROCEDURE – RAW MATERIAL					
Material	**Sodium chloride**				
Item Code	**1000001486**			**Department**	**Quality Control**
STP No.	RLS2/QC/RM/STP016	**Revision**	00	**Effective Date**	Jun 19, '07
Supersedes	None	**Dated**	NA	**Page No.**	Page 1 of 19
Next Review on	Maximum of 2 years from effective date				

01	Characters	A white, crystalline powder or colourless crystals or white pearls, freely soluble in water, practically insoluble in ethanol.	BP
	Description	Colourless crystals or white, crystalline powder.	IP

Apparatus:

100 ml measuring cylinder

Clear Petri dish

Plain white paper

Clean glass test tubes

Water

Ethanol

Procedure:

1) Take 5 g of sample in a clear Petri dish. Place a plain white paper in a well-cleaned area and place the Petri dish on it, record observations about the following descriptive properties.
    a) Color (White, off-white, yellow, etc).
    b) Physical state (Crystalline, amorphous, etc.)
2) Take
    a) 1g of the sample in a clean glass test tube, add 10 ml of water to it and record the solubility of sample with water. (Freely soluble)
    b) 0.01g of sample in a clean glass test tube, add 100 ml of ethanol to it and record the solubility of sample. (Practically insoluble)

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

02	Solubility	Freely soluble in water and slightly more soluble in boiling water, practically insoluble in ethanol.	IP

Apparatus and chemicals:

Procedure:

Follow the procedure mentioned in Test No. 01.

03	Identification	A. Gives the reaction of chlorides. B. Gives the reaction of sodium (salts).	IP/BP

A.

Apparatus and Chemicals:

Analytical balance

Water

2M nitric acid

Silver nitrate

10M ammonia

Potassium dichromate

Sulphuric acid

Filter paper

Diphenylcarbazide

Procedure:

1) Dissolve a quantity of the substance being examined containing about 2 mg of chloride in 2 ml of water. Acidify the solution with 2M nitric acid, add 0.4 ml of silver nitrate solution, shake and allow to stand; (a curdy, white precipitate is produced). Centrifuge and wash the precipitate with three 1-ml quantities of water. Carry out this operation rapidly in subdued light, disregarding the fact that the supernatant solution may not become perfectly clear. Suspend the precipitate in 2 ml of water and add 1.5 ml 10M ammonia; the precipitate dissolves easily with the possible exception of a few large particles which dissolve slowly.
2) Introduce into a test tube a quantity of the substance being examined, add 0.2 g of potassium dichromate and 1 ml of sulphuric acid and place a filter paper strip moistened with 0.1 ml of diphenylcarbazide solution over the opening of the test tube. The paper turns violet-red. The moistened paper must not come into contact with the potassium dichromate solution.

B.

Apparatus and Chemicals:

Analytical balance

Test tube

Potassium carbonate

Potassium antimonite solution

Methoxyphenylacetic acid reagent

6M ammonia

Ammonium carbonate

Procedure:

1) To 0.1 g of the substance to be examined add 2 ml of water, add 2 ml of a 15% w/v solution of potassium carbonate and heat to boiling; no precipitate is produced. Add 4 ml of freshly prepared potassium antimonate solution and heat to boiling. Allow to cool in ice and if necessary scratch the inside of the tube with a glass rod. A dense, white precipitate is produced.
2) To 0.5 ml of a solution containing about 2 mg of sodium ion add 1.5 ml of methoxyphenylacetic acid reagent and cool in ice for 30 minutes; a voluminous, white, crystalline precipitate is produced. Warm in water at 20° and stir for 5 minutes; the precipitate does not dissolve. Add 1 ml of 6M ammonia; the precipitate dissolves completely. Add 1 ml of 16% w/v solution of ammonium carbonate; no precipitate is produced.

04	Appearance of solution	Clear and colourless	BP
	Clarity and colour of solution	Clear and colourless	IP

Apparatus and Chemicals:

2 identical tubes of colourless, transparent, neutral glass with a flat base and internal diameter of 15 to 25 mm

Water

Procedure:

Solution S: Dilute 20 g of sample to 100 ml with distilled water.

Compare the appearance of Solution S using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm compare a 40-mm layer of the liquid being examined with a 40-mm layer of water. Examine the columns of liquid in diffused daylight by viewing down the vertical axes of the tubes against a white background. The liquid is clear if its clarity is the same as that of water.

05	Acidity or alkalinity	Not more than 0.5ml of 0.01M hydrochloric acid or of 0.01M sodium hydroxide is required.	BP
		Not more than 0.5ml of 0.01M hydrochloric acid or of 0.01M sodium hydroxide is required.	IP

Apparatus and Chemicals:

Bromothymol blue solution

0.01M Hydrochloric acid

0.01M Sodium hydroxide

Procedure:

Take 10 ml of Solution S and add 0.1 ml of bromothymol blue solution. Not more than 0.5 ml of 0.01M hydrochloric acid or 0.01M sodium hydroxide is required to change the colour of the indicator.

06	Arsenic	Complies with the limit test for arsenic(1ppm)	IP/BP

Apparatus and Chemicals:

Arsenic apparatus

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

250 ml conical flask

100 ml conical flask

Stanned hydrochloric acid

1M potassium iodide

Zinc AsT

Arsenic trioxide

2M sodium hydroxide

Procedure:

Dissolve 10 g of substance to be examined in 50 ml of water and 12 ml of stanned hydrochloric acid AsT. Into the conical flask of arsenic apparatus introduce the solution prepared, add 5 ml of 1M potassium iodide and 10 g of zinc AsT. Immediately assemble the apparatus and immerse the flask in a water-bath at a temperature such that a uniform evolution of gas is maintained. After 40 minutes any stain produced on the mercuric chloride paper is not more intense than that obtained by treating in the same manner 1.0 ml of arsenic standard solution (10 ppm As) diluted to 50 ml with water.

Preparation of Arsenic Standard solution;

Dissolve 0.33 g of arsenic trioxide in 5 ml of 2M sodium hydroxide and dilute to 250.0 ml of water. Dilute 1 ml of the solution to 100ml with water (10 ppm As). Dilute 10 ml of the 10 ppm solution to 100 ml with water (1 ppm).

07	Magnesium and alkaline-earth metals	Not more than 100 ppm (calculated as Ca)	BP
	Calcium and Magnesium	Not more than 50 ppm.	IP

Apparatus:

500 ml measuring cylinder, analytical balance, ammonium chloride buffer solution, mordant black 11 triturate, 0.05M zinc chloride, 0.02M sodium edentate.

Procedure:

To 200 ml of water add 0.1 g of hydroxylamine hydrochloride, 10 ml of ammonia buffer pH 10.0, 1 ml of 0.1M zinc sulphate and about 15 mg of mordant black 11 triturate. Heat to about 40 °C and titrate with 0.01M disodium edetate until the violet colour changes to a full blue. To the solution add 10 g of the substance to be examined dissolved in 100 ml of water. If the colour of the solution changes to violet, titrate with 0.01M disodium edetate until the full blue colour is again produced. The volume of 0.01M disodium edetate used in the second titration does not exceed 2.5 ml.

08	Iron	Complies with the limit test for iron (2ppm)	BP
		Complies with the limit test for iron (20ppm)	IP

Apparatus and Chemicals:

Nessler cylinder

Analytical balance

Citric acid

Mercaptoacetic acid

10M ammonia

Water

Ammonium iron (III) sulphate

1M sulphuric acid

Procedure:

Take 10 ml of Solution S and transfer to a Nessler cylinder. Add 2 ml of a 20% w/v solution of citric acid and 0.1 ml of mercaptoacetic acid, mix, make alkaline with 10M ammonia, dilute to 20 ml with water and allow to stand for 5 minutes. Any pink color produced is not more intense than that obtained by treating 10 ml of iron standard solution (1 ppm Fe) in the same manner.

Preparation of Iron standard solution:

Dissolve 0.863 g of ammonium iron (III) sulphate in water containing 25 ml of 1M sulphuric acid and add sufficient water to produce too ml. Dilute 1 ml to 10ml with water. Take 1 ml of the above solution and dilute to 20 ml with water immediately before use. Take 4 ml of the solution and mix 6 ml of water.

09	Barium	Opalescence in the solution is not more than that in a mixture of 5 ml of solution and distilled water.	BP
		No turbidity is produced within 2 hours.	IP

Apparatus and chemicals:

100 ml conical flask

10 ml Pipette

Sulphuric acid

Distilled water

Procedure:

To 5 ml of Solution S add 5 ml of distilled water and 2 ml of dilute sulphuric acid. After 2 h, any opalescence in the solution is not more intense than that in a mixture of 5 ml of test solution and 7 ml of distilled water.

10	Bromide	The absorbance of the solution in not greater than that of standard (50 ppm).	BP
		Any violet colour produced is not more intense than that of standard.	IP

Apparatus and chemicals:

Analytical balance

100 ml volumetric flask

Water

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

Chloramine solution

0.1M sodium thiosulphate

Potassium bromide

Procedure:

Dissolve 1.0 g in sufficient water to produce 100.0 ml. To 0.5 ml of the solution add 9 ml of water, 1 ml of phenol red solution and 0.05 ml of chloramine solution, shake for 15 seconds and add 0.15 ml of 0.1M sodium thiosulphate. Any violet colour produced is not more intense than that of a solution prepared at the same time and in the same manner using 0.5 ml of a 0.0015% w/v solution of potassium bromide and 9 ml of water.

11	Heavy metals	Complies with limit test for heavy metals (5 ppm).	BP
		Not more than 5ppm	IP

Apparatus and Chemicals:

Measuring cylinder

Water

Acetate buffer pH 3.5

Thioacetamide reagent

Lead nitrate

Procedure:

Preparation of Test solution:

To 12 ml of Solution S add 2 ml of acetate buffer pH 3.5, mix, add 1.2 ml of thioacetamide reagent, mix immediately and allow to stand for 2 minutes. Any brown

colour produced is not more intense than that obtained by treating in the same manner a mixture of 10 ml of lead standard solution (1 ppm Pb) and 2 ml of the solution being examined. The standard solution exhibits a slightly brown colour when compared to a solution prepared by treating in the same manner a mixture of 10 ml of water and 2 ml of the solution being examined.

Preparation of Lead standard solution:

Take 0.4 g of lead nitrate, dilute to 250 ml with water (to produce 0.1% Pb). Dilute 1 volume of the solution to 100 volumes with water (to produce 10 ppm Pb). Dilute 1 volume of the 10 ppm Pb solution to 10 volumes with water immediately before use to get 1 ppm Pb solution.

12	Ferrocynide	No blue colour develops within 10 min.	BP
		No blue colour is produced within 10 minutes	IP

Chemicals and Apparatus:

Analytical balance

Ferric ammonium sulphate

Sulphuric acid

Ferrous sulphate

Water

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

Procedure:

Dissolve 2.0 g in 6 ml of water and add 0.5 ml of a mixture of 5 ml of a 1% w/v solution of ferric ammonium sulphate in a 0.25% w/v solution of sulphuric acid, and 95 ml of a 1% w/v solution of ferrous sulphate.

13	Iodide	The substance shows no blue colour.	BP
		The substance shows no blue colour after 5 minutes.	IP

Chemicals and Apparatus:

Analytical balance

Sodium nitrite

0.5M sulphuric acid

Iodide-free starch solution

Water

Procedure:

Moisten 5 g by dropwise addition of a freshly prepared mixture of 0.15 ml of sodium nitrite solution, 2 ml of 0.5M sulphuric acid, 25 ml of iodide-free starch solution and 25 ml of water. After 5 min, examine in daylight.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

14	Loss on drying	Not more than 0.5 per cent.	BP
		Not more than 1.0%.	IP

Apparatus:

Analytical balance

Vacuum oven

Petri dish

Desiccator

Thermometer

Procedure:

Take empty Petri dish and dry in vacuum oven at 100 °C for ½ hour and remove the Petri dish from drier, cool to room temperature and record the weight (W1). Weigh 1.0 g of sample and transfer it into Petri dish record the weight (W2). Keep the Petri dish in oven and dry the material under vacuum at 100° to 105°C. Remove the Petri dish and cool to room temperature in a desiccator and record the weight (W3). Dry the material till the two successive weights will be constant (The variation for two successive weights allowed is 0.5 mg).

Calculate the LOD of the sample as given below

$$\text{LOD of the sample} = \frac{W2 - W3}{W2 - W1} \times 100 = \quad \%$$

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

15	Phosphates	Complies with the limit test for phosphates (25 ppm).	BP

Apparatus and chemicals:

100 ml volumetric flask

Sulphomolybdic reagent

Tin(III) chloride solution

Potassium dihydrogen orthophosphate

Water

Procedure:

Dilute 2 ml of Solution S to 100 ml with water, add 4 ml of sulphomolybdic reagent, shake, add 0.1 ml of tin (III) chloride solution, allow to stand for 10 minutes and examine 20 ml of the resulting solution. Any colour produced is not more intense than that produced in 20 ml of a solution obtained by treating a mixture of 2 ml of phosphate standard solution (5 ppm PO_4) and 98 ml of water in the same manner.

Preparation of phosphate standard solution:

Take 0.0716 g of potassium dihydrogen orthophosphate; dissolve in 100 ml of water. Dilute 1 ml of the solution to 100 ml with water immediately before use.

16	Sulphates	Complies with the limit test for sulphates (200ppm)	BP
		Complies with the limit test for sulphates (300ppm)	IP

Chemical and Apparatus:

Nessler cylinder

Analytical balance

100 ml volumetric flask

Barium chloride

5M acetic acid

Potassium sulphate

Ethanol (30%)

Procedure:

Dilute 7.5 ml of Solution S to 30 ml with distilled water.

Add 1.0 ml of a 25.0% w/v solution of barium chloride to 1.5 ml of sulphate standard solution (10 ppm SO_4), shake and allow to stand for 1 minute. Add 15 ml of the above test solution and 0.5 ml of 5M acetic acid and allow to stand for 5 minutes. Any opalescence produced is not more intense than that of a standard prepared in the same manner but using 15 ml of sulphate standard solution (10 ppm SO_4) in place of the solution being examined.

Preparation of Sulphate standard solution:

Take 0.181 g of potassium sulphate in ethanol (30%). Dilute 1 ml of the solution to 100 ml with ethanol (30%).

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

17.	Aluminium	Not more than 0.2 ppm.	IP/BP

Apparatus and chemicals:

Analytical balance

100 ml measuring jar

8-hydroxyquinoline

Chloroform

Acetate buffer pH 6.0

Water

Aluminium potassium sulphate

0.1M sulphuric acid

Procedure:

Dissolve 20.0 g in 100 ml of water and add 10 ml of acetate buffer solution pH 6.0. Extract the above solution with successive quantities of 20, 20 and 10ml of 0.5% w/v solution of 8-hydroxyquinoline in chloroform and dilute the combined extracts to 50 ml with chloroform. Use as blank solution a mixture of 10 ml of acetate buffer pH 6.0 and 100 ml of water treated in the same manner and as the standard solution a mixture of 2 ml of aluminium standard solution (2 ppm Al), 10 ml of acetate buffer pH 6.0 and 98 ml of water treated in the same manner. Measure the fluorescence of the test solution (I_1), of the standard solution(I_2) and of the blank(I_3), using and excitation wavelength of 392 nm and a secondary filter with a transmission band centred at 518 nm, or a monochromator set to transmit at this wavelength. The fluorescence of the test solution. ($I_1 - I_3$) is not greater than that of the standard solution ($I_2 - I_3$).

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

Preparation of Aluminium standard solution:

Take 0.352 g of aluminium potassium sulphate and dissolve in 100 ml of 0.1M sulphuric acid. Take 1 ml of the resulting solution and dilute 100 ml with water immediately before use.

18.	Nitrites	The absorbance is not greater than 0.01.	BP

Chemicals and apparatus:

UV-VIS Spectrophotometer

Water

Procedure:

To 10 ml of Solution S add 10 ml of water. Measure the absorbance of the solution at 354 nm.

19.	Potassium (flame photometry)	Contains not more than 500 ppm of K	BP
		Not more than 0.1%	IP

Apparatus and chemicals:

Analytical balance

Potassium chloride

Water

Vacuum oven

Atomic emission spectrometer

Procedure:

Prepare the test solution as given below. Prepare the reference solutions of the element being determined with concentrations of 400 ppm, 500 ppm, 600 ppm, 900 ppm, 1000 ppm and 1100 ppm. Introduce the test solution and each reference solution into the instrument three times and record the steady reading. Rinse the apparatus with blank solution each time and ascertain that the reading returns to its initial blank value. Prepare a calibration curve from the mean of the readings obtained with the reference solutions and determine the concentration of the element in the test solution from the curve so obtained.

Measure the emission intensity at 766.5 nm.

Preparation of Test solution:

Dissolve 1.00 g of the substance to be examined in water and dilute to 100.0 ml with the same solvent.

Preparation of Reference solutions:

Dissolve 1.144 g of potassium chloride, previously dried at 100-105°C for 3 h, in water and dilute to 1000.0 ml with the same solvent (600 µg of K per milliliter).

20	Bacterial endotoxins	Less than 5 IU/g.	BP

Send the sample to Microbiology department for the analysis of Bacterial endotoxins.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

21	Assay (Dried Substance)	Not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of NaCl	BP
		Not less than 99.0 per cent and not more than 100.5 per cent of NaCl.	IP

Apparatus and chemicals:

Analytical balance, Burette, conical flask, water, nitric acid, 0.1M silver nitrate, dibutyl phthalate, 0.1M ammonium thiocyanate, ferric ammonium sulhate indicator

Procedure:

Dissolve 1.000 g of substance to be examined in water and dilute to 100 ml with the same solvent. To 10.0 ml of the solution add 50 ml of water, 5 ml of dilute nitric acid, 25.0 ml of 0.1M silver nitrate and 2 ml of dibutyl phthalate. Shake. Titrate with 0.1M ammonium thiocyanate, using 2 ml of ferric ammonium sulphate solution as indicator and shaking vigorously towards the endpoint.

Calculate the assay of NaCl using the following formula:

$$\text{Assay} = \frac{\text{Titer value} \times \text{Molarity of ammonium thiocyanate} \times 5.844 \times 100}{\text{Weight of the sample take in mg} \times 0.1}$$

$$= \quad \%$$

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

ANALYTICAL TEST REPORT

Product Information

Material Sodium chloride
Item Code : 1000001486
Specification No. RLS2/QC/RM/SPC016-00
GRN No. :
ATR No. RLS2/QC/RM/ATR016-00
AR No. :

Sampling Information

Collected By
Receipt Date :

Analysis Information

S. No.	Parameters	Result	Specification	Ref
01).	Characters		A white, crystalline powder or colourless crystals or white pearls, freely soluble in water, practically insoluble in ethanol.	BP
	Description		Colourless crystals or white, crystalline powder.	IP
02).	Solubility		Freely soluble in water and slightly more soluble in boiling water, practically insoluble in ethanol.	IP
03).	Identification		A. It gives the reaction of chlorides. B. It gives the reaction of sodium.	BP
			A. Gives the reaction of chlorides. B. Gives the reaction of sodium (salts).	IP
04).	Appearance of solution		Clear and colourless	BP
	Clarity and colour of solution		Clear and colourless	IP
05).	Acidity or alkalinity		Not more than 0.5ml of 0.01M hydrochloric acid or of 0.01M sodium hydroxide is required	BP
			Not more than 0.5ml of 0.01M hydrochloric acid or of 0.01M sodium hydroxide is required.	IP
06).	Arsenic		Complies with the limit test for arsenic(1ppm)	BP
07).	Magnesium and alkaline-earth metals		Complies with the limit test for magnesium and alkaline-earth metals.	BP
	Calcium and Magnesium		Not more than 50 ppm.	IP

Result : Sample meets / Does not meets the requirements as per specification.

Analysed By :
Date :

ANALYTICAL TEST REPORT

Product Information

Material Sodium chloride
Specification No. RLS2/QC/RM/SPC016-00
ATR No. RLS2/QC/RM/ATR016-00
Item Code : 1000001486
GRN No. :
AR No. :

Sampling Information

Collected By
Receipt Date :

Analysis Information

S. No.	Parameters	Result	Specification	Ref
08).	Iron		Complies with the limit test for iron (2ppm).	IP
			Complies with the limit test for iron (20ppm).	BP
09)	Barium		Opalescence in the solution is not more than that in a mixture of 5 ml of solution and distilled water.	BP
			No turbidity is produced within 2 hours	IP
10)	Bromide		The absorbance of the solution is not greater than that of standard (50 ppm).	IP
			Any violet colour produced is not more intense than that of standard.	BP
11)	Heavy metals		Complies with limit test for heavy metals (5 ppm).	BP
			Not more than 5ppm	IP
12)	Ferrocynide		No blue colour develops within 10 min	BP
			No blue colour is produced within 10 minutes.	IP
13)	Iodide		The substance shows no blue colour.	BP
			The substance shows no blue colour after 5 minutes.	IP
14)	Loss on drying:		Not more than 0.5 per cent.	BP
			Not more than 1.0%.	IP
15)	Phosphates		Complies with the limit test for phosphates(25 ppm) .	BP

Result : Sample meets / Does not meets the requirements as per specification.

Analysed By :
Date :
By :
Date :

ANALYTICAL TEST REPORT

Product Information

Material: Sodium chloride
Specification No.: RLS2/QC/RM/SPC016-00
ATR No.: RLS2/QC/RM/ATR016-00
Item Code: 1000001486
GRN No.:
AR No.:

Sampling Information

Collected By:
Receipt Date:

Analysis Information

S. No.	Parameters	Result	Specification	Ref
16)	Sulphates		Complies with the limit test for sulphates (200ppm)..	BP
			Complies with the limit test for sulphates (300 ppm).	IP
17)	Aluminium		Complies with the test for aluminium(0.2 ppm)	BP
			Not more than 0.2 ppm.	IP
18)	Nitrites		The absorbance is not greater than 0.01.	BP
19)	Potassium		Contains not more than 500 ppm of K.	BP
			Not more than 0.1%, determined by flame photometry.	IP
20)	Bacterial endotoxins		Less than 5 IU/g.	BP
21)	Assay		Not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of NaCl	BP
			Not less than 99.0 per cent and not more than 100.5 per cent of NaCl.	IP

Result : Sample meets / Does not meets the requirements as per specification.

Analysed By :
Date :

ANALYTICAL TEST REPORT

Product Information

Material: Sodium chloride (B123R07)
Specification No.: RLS2/QC/RM/SPC016-00
ATR No.: RLS2/QC/RM/ATR016-00
Item Code: 1000001486
GRN No.: 5006913571
AR No.: AR/RM/08/5031

Sampling Information

Collected By: _____
Receipt Date: 15-03-08

Analysis Information

S. No.	Parameters	Result	Specification	Ref
01).	Characters	A white crystalline powder. Solubility Complies	A white, crystalline powder or colourless crystals or white pearls, freely soluble in water, practically insoluble in ethanol.	BP
	Description	White crystalline powder	Colourless crystals or white, crystalline powder.	IP
02).	Solubility	Complies	Freely soluble in water and slightly more soluble in boiling water, practically insoluble in ethanol.	IP
03).	Identification	Complies Complies	A. It gives the reaction of chlorides. B. It gives the reaction of sodium.	BP
		Complies Complies	A. Gives the reaction of chlorides. B. Gives the reaction of sodium (salts).	IP
04).	Appearance of solution	Clear and Colourless	Clear and colourless	BP
	Clarity and colour of solution	Clear and Colourless	Clear and colourless	IP
	[illegible]dity or alkalinity	Complies	Not more than 0.5ml of 0.01M hydrochloric acid or of 0.01M sodium hydroxide is required	BP
		Complies	Not more than 0.5ml of 0.01M hydrochloric acid or of 0.01M sodium hydroxide is required.	IP
06).	Arsenic	Complies	Complies with the limit test for arsenic(1ppm)	BP
07).	Magnesium and alkaline-earth metals	Complies	Complies with the limit test for magnesium and alkaline-earth metals.	BP
	Calcium and Magnesium	Complies	Not more than 50 ppm.	IP

Result : Sample meets / ~~Does not meets~~ the requirements as per specification. (IP Only)
(Material not to be used as per BP specifications.)

Analysed By :
Date :

Reviewed By :
Date :

Repeat test Per AR10M/no/12540

ANALYTICAL TEST REPORT ... ST REPORT

Product Information

Material: Sodium chloride (B123K07)
Specification No.: RLS2/QC/RM/SPC016-00
ATR No.: RLS2/QC/RM/ATR016-00

Item Code: 1000001486
GRN No.: 5006913571
AR No.: AR/RM/08/5031

Sampling Information

Collected By: _____
Receipt Date: 15.03.08

Analysis Information

S. No.	Parameters	Result	Specification	Ref
08).	Iron	Complies	Complies with the limit test for iron (2ppm).	IP
		Complies	Complies with the limit test for iron (20ppm).	BP
09)	Barium	Complies	Opalescence in the solution is not more than that in a mixture of 5 ml of solution and distilled water.	BP
		Complies	No turbidity is produced within 2 hours	IP
10)	Bromide	Complies	The absorbance of the solution is not greater than that of standard (50 ppm).	IP
		Complies	Any violet colour produced is not more intense than that of standard.	BP
11)	Heavy metals	Complies	Complies with limit test for heavy metals (5 ppm).	BP
		Complies	Not more than 5ppm	IP
12)	Ferrocynide	Complies	No blue colour develops within 10 min	BP
		Complies	No blue colour is produced within 10 minutes.	IP
	Iodide	Complies	The substance shows no blue colour.	BP
		Complies	The substance shows no blue colour after 5 minutes.	IP
14)	Loss on drying:	0.2%	Not more than 0.5 per cent.	BP
		0.2%	Not more than 1.0%.	IP
15)	Phosphates	Complies	Complies with the limit test for phosphates(25 ppm).	BP

Result: Sample meets / ~~Does not meets~~ the requirements as per specification: (IP Only)
(Material not to be used as per BP specifications).

Analysed By:
Date:

Reviewed By:
Date:

ANALYTICAL TEST REPORT FOR IP

Product Information

Material: Sodium chloride (B123K07)
Specification No.: RLS2/QC/RM/SPC016-00
ATR No.: RLS2/QC/RM/ATR016-00

Item Code: 1000001486
GRN No.: 5006913571
AR No.: AR/RM/08/5031

Sampling Information

Collected By: R.P Patil
Receipt Date: 15.03.08

Analysis Information

S. No.	Parameters	Result	Specification	Ref
16)	Sulphates	Complies	Complies with the limit test for sulphates (200ppm)..	BP
		Complies	Complies with the limit test for sulphates (300 ppm).	IP
17)	Aluminium	Complies	Complies with the test for aluminium(0.2 ppm)	BP
		Complies	Not more than 0.2 ppm.	IP
18)	Nitrites	Complies	The absorbance is not greater than 0.01.	BP
19)	Potassium	Not Done	Contains not more than 500 ppm of K.	BP
		Complies	Not more than 0.1%, determined by flame photometry.	IP
20)	Bacterial endotoxins	Complies	Less than 5 IU/g.	BP
21)	Assay	99.6%	Not less than 99.0 per cent and not more than the equivalent of 100.5 per cent of NaCl	BP
		99.6%	Not less than 99.0 per cent and not more than 100.5 per cent of NaCl.	IP

1 Material not to be used as per BP specifications

Result: Sample meets / ~~Does not meets~~ the requirements as per specification.

Analysed By:
Date:

Reviewed By:
Date:

(Refer ATR for AR/RM/08/3538)

RFCL LIMITED

CERTIFICATE OF ANALYSIS

Name of Product : SODIUM CHLORIDE IP-50 KG **Batch No.** : B123K07

Inspection Lot :10000057718 **Specification No.** : FCS-019

TEST	RESULTS	SPECIFICATION
DESCRIPTION	Complies	Colourless crystals or white crystalline powder.
SOLUBILITY	Complies	Freely soluble in water and slightly more soluble in boiling water, practically insoluble in ethanol.
IDENTIFICATION	Complies	Passes Test
ACIDITY/ALKALINITY	Complies	Passes Test
CLARITY AND COLOUR OF SOLUTION	Complies	Passes Test
ARSENIC(As)	< 0.0001%	<=0.0001%
BARIUM (Ba)	Complies	Passes Test
BROMIDE	Complies	Passes Test
CALCIUM (Ca)& MAGNESIUM	< 0.005%	<=0.005%
FERROCYANIDE	Complies	Passes Test
HEAVY METAL (as Pb)	< 0.0005%	<=0.0005%
IODIDE	Complies	Passes Test
IRON (Fe)	< 0.0020%	<=0.002%
LOSS ON DRYING	0.19 %	<= 1.00 %
ASSAY	99.53 %	>= 99.00 %

Tested By **Approved By**

(Quality Assurance Chemist) **(Quality Assurance Manager)**

This is an electronically generated document and is valid without a signature .

RFCL LIMITED

CERTIFICATE OF ANALYSIS

Name of Product : SODIUM CHLORIDE IP-50 KG **Batch No.** : B123K07

Inspection Lot :10000057718 **Specification No.** : FCS-019

TEST	RESULTS	SPECIFICATION
POTASSIUM(K)	< 0.1%	<=0.1%
ALUMINIUM(Al)	< 0.00002%	<=0.00002%

Remark:- The batch complies with the laid down specification.
APPROVED.

Tested By **Approved By**

(Quality Assurance Chemist) **(Quality Assurance Manager)**

This is an electronically generated document and is valid without a signature

SPECIFICATION – RAW MATERIAL

Material	**Sodium hydroxide IP/BP/USP-NF**				
Item Code	**1000002770**			**Department**	**Quality Control**
Specification No.	RLS2/QC/RM/SPC018	**Revision**	01	**Effective Date**	11 Jan 08
Supersedes	00	**Dated**	19 Jun 07	**Page No.**	Page 1 of 3
Next Review on	Maximum of 2 years from effective date				

Storage conditions	Preserve in tight containers.	USP
	Store in an airtight, non-metallic container.	BP
	Store in tightly-closed, non-metallic containers.	IP
Re-testing period	One year	
Sample Quantity	10g + (Retention sample : 2 × 10g)	
Critical raw material	Yes	

S. No.	Test	Specifications	Ref
01	Characters	White, crystalline masses, supplied as pellets, sticks or slabs, deliquescent, readily absorbing carbon dioxide, very soluble in water, freely soluble in alcohol.	BP
	Description	White, crystalline masses supplied as sticks, pellets or slabs; deliquescent. Readily absorbs carbon dioxide.	IP
02	Solubility	Very soluble in water, freely soluble in ethanol (95%).	IP
03	Identification	A solution (1 in 25) responds to the tests for Sodium	USP
		A. pH of the solution is not less than 11.0 B. Gives reaction of sodium.	BP
		A. Gives reaction of sodium salts. B. pH of a 0.01% w/v solution, not less than 11.0	IP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION – RAW MATERIAL

Material	**Sodium hydroxide IP/BP/USP-NF**				
Item Code	**1000002770**			**Department**	**Quality Control**
Specification No.	RLS2/QC/RM/SPC018	**Revision**	01	**Effective Date**	11 Jan 08
Supersedes	00	**Dated**	19 Jun 07	**Page No.**	Page 2 of 3
Next Review on	Maximum of 2 years from effective date				

S. No.	Test	Specifications	Ref
04	Insoluble substances and organic matter	Clear, and colorless to slightly colored.	USP
	Appearance of solution	Clear, and colourless.	BP
	Clarity and colour of solution	Clear, and colourless.	IP
05	Potassium	No precipitate is formed	USP
06	Carbonates	Not more than 2.0 per cent.	BP
07	Chlorides	Complies with the limit test for chlorides (50 ppm).	BP
		Complies with the limit test for chlorides (125 ppm).	IP
08	Sulphates	Complies with the limit test for sulphates (50 ppm).	BP
		Complies with the limit test for sulphates (75 ppm).	IP
09	Iron	Complies with the limit test for iron (10 ppm).	BP
		Complies with the limit test for iron (20 ppm).	IP
10	Heavy metals	The limit is 0.003%.	USP
		Complies with limit test for heavy metals (20 ppm).	BP
		Not more than 20 ppm.	IP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION – RAW MATERIAL					
Material	**Sodium hydroxide IP/BP/USP-NF**				
Item Code	**1000002770**			**Department**	**Quality Control**
Specification No.	RLS2/QC/RM/SPC018	**Revision**	01	**Effective Date**	11 Jan 08
Supersedes	00	**Dated**	19 Jun 07	**Page No.**	Page 3 of 3
Next Review on	Maximum of 2 years from effective date				

S. No.	Test	Specifications	Ref
11	Arsenic	Complies with the limit test for arsenic (4 ppm).	IP
12	Assay	Not less than 97.0 per cent and not more than the equivalent of 100.5 per cent of total alkali, calculated as NaOH.	BP/IP
		Not less than 95.0 per cent and not more than the equivalent of 100.5 per cent of total alkali, calculated as NaOH. Including 3% Na_2CO_3	USP

Parameters for re-test:

S. No.	Test	Specifications	Ref
01	Characters	White, crystalline masses, supplied as pellets, sticks or slabs, deliquescent, readily absorbing carbon dioxide, very soluble in water, freely soluble in alcohol.	BP
	Description	White, crystalline masses supplied as sticks, pellets or slabs; deliquescent. Readily absorbs carbon dioxide.	IP
02	Assay	Not less than 97.0 per cent and not more than the equivalent of 100.5 per cent of total alkali, calculated as NaOH.	BP/IP
		Not less than 95.0 per cent and not more than the equivalent of 100.5 per cent of total alkali, calculated as NaOH. Including 3% Na_2CO_3	USP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

STANDARD TESTING PROCEDURE – RAW MATERIAL					
Material	**Sodium hydroxide**				
Item Code	**1000002770**			**Department**	**Quality Control**
STP No.	RLS2/QC/RM/STP018	**Revision**	00	**Effective Date**	Jun 13, '07
Supersedes	None	**Dated**	NA	**Page No.**	Page 1 of 11
Next Review on	Maximum of 2 years from effective date				

01	Characters	White, crystalline masses, supplied as pellets, sticks or slabs, deliquescent, readily absorbing carbon dioxide, very soluble in water, freely soluble in alcohol.	BP
	Description	White, crystalline masses supplied as sticks, pellets or slabs; deliquescent. Readily absorbs carbon dioxide.	IP

Apparatus and Chemicals:

Clean Petri dish

Plain white paper

Procedure:

Take about 5 g of sample in a clean and dry Petri dish. Place a plain white paper in a well-cleaned area; place the Petri dish on it and record observations about the following descriptive properties.

a) Colour (Clear, Colourless, yellow, etc.)

b) Physical state (Crystalline, amorphous, odour, etc.)

02	Solubility	Very soluble in water, freely soluble in ethanol (95%).	IP

Apparatus and chemicals:

Clean glass test tubes

Water

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

Ethanol (95%)

Procedure:

Take

a) 1 g of sample and add 10 ml of water (Very soluble).

b) 0.1 g of sample and add 100 ml of ethanol-95% (Freely soluble)

03	Identification	A. Gives reaction of sodium. B. pH of the final solution is not less than 11.0	BP
		A. Gives reaction of sodium salts. B. pH of a 0.01% w/v solution, not less than 11.0	IP

A-BP/B-IP:

Apparatus and chemicals: (Sodium)

Analytical balance

10ml measuring cylinder

Water

Potassium carbonate

Potassium antimonite

Ice

Water bath

Procedure:

Take 3.0 g of sample and add 6ml of distilled water, adjust to pH 7 with hydrochloric acid (about 7.5 ml) and dilute to 15 ml with distilled water. Take 2 ml of the solution and add 2 ml of a 15% w/v solution of potassium carbonate and heat to boiling; no precipitate is produced. Add 4 ml of freshly prepared potassium antimonate solution and heat to boiling. Allow to cool in ice and if necessary scratch the inside of the tube with a glass rod. A dense, white precipitate is produced.

B-BP/A-IP:

Apparatus and Chemicals:

pH meter

100 ml beaker

Carbon dioxide-free water

Analytical balance

Procedure:

Take 1.0 g of sample and add 30 ml of carbon dioxide-free water. Immerse the electrodes in the solution and measure the pH at the same temperature as for the standard solutions. At the end of a set of measurements, record the pH of the solution used to standardize the meter and electrodes. Repeat the measurements, If the difference between this reading and the original value is greater than 0.05.

04	Appearance of solution	Clear, and colourless.	BP
	Clarity and colour of solution	Clear, and colourless.	IP

Apparatus and Chemicals:

2 identical tubes of colourless, transparent, neutral glass with a flat base and internal diameter of 15 to 25 mm

Water

Procedure:

Dilute 1.0 g of sample to 10 ml with water. Compare the appearance of the solution using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15 to 25 mm compare a 40-mm layer of the liquid being examined with a 40-mm layer of water. Examine the columns of liquid in diffused daylight by viewing down the vertical axes of the tubes against a white background. The liquid is clear if its clarity is the same as that of water.

05	Carbonates	Not more than 2.0 per cent	BP

Apparatus and Chemicals:

Procedure:

Calculate as Na_2CO_3, as determined in the assay.