SUMMARY REPORT FORM
FOR
VERIFICATION STUDIES AND SYSTEM VALIDATION DATA

LABORATORY SECTION: HIV – 1 RNA Quantification

OLD METHODOLOGY: HIV – 1 RNA Quantification using manual extraction

NEW METHODOLOGY: HIV – 1 RNA Quantification using the Ampliprep System

Date New Instrument/Test Kit Received: February 2006

Date New Instrument Installed: February 2006

Instrument Serial Number:391527

VERIFICATION STUDIES:
The Accuracy testing was performed using 20 randomised Edta plasma samples that were tested in duplicate using the Manual extraction method and the Ampliprep extraction Method.

1. Precision

Testing for Precision was performed from 20 April 2006 to 24 May 2006 and included 20 valid runs run on the same batch number of Low Positive (G10827) and High Positive (G10825).

	LOW POSITIVE CONTROL Range:910 - 8200 Copies/ml			HIGH POSITIVE CONTROL Range:8300 - 75000 Copies/ml	
	Value Copies/ml	Log		Value Copies/ml	Log
1	3080	3.49		34800	4.54
2	5840	3.77		25000	4.39
3	3730	3.57		40600	4.61
4	2270	3.36		38400	4.58
5	1860	3.27		19900	4.29
6	2750	3.44		30300	4.48
7	4440	3.65		19900	4.29
8	2690	3.43		21400	4.33
9	3200	3.51		28400	4.45
10	2200	3.34		13400	4.13
11	2740	3.44		21500	4.33
12	2810	3.45		23400	4.37
13	1310	3.12		17000	4.23
14	3020	3.48		16100	4.21
15	1790	3.25		15600	4.19
16	2000	3.3		27500	4.44
17	1700	3.23		19800	4.29

18	1860	3.27		19900	4.29
19	2210	3.34		14800	4.17
20	3080	3.49		19500	4.29
CV	38.46	4.51		33.22	3.13

2. **Linearity**

The linearity of the kit is stated from 400 copies/mL to 750 000 copies/mL. This range has been demonstrated in the CAPRISA Research Laboratory on many previous instrument runs and is reflected again in the "Accuracy" table below. Patient samples were selected which had results covering the entire testing range (i.e. <400 copies/mL, mid-range, high values (493 000 copies/mL) and finally samples with more than 750 000 copies/mL). Results obtained consistently demonstrated the ability of the kit to detect sample values throughout the stated range of detection with no anomalies.

Samples with results greater than 750 000 copies/mL were diluted with Negative Human Plasma (NHP) and retested. In all cases the results obtained once multiplied by the dilution factor were greater than 750 000 copies/mL.

The highest result we have demonstrated has been a sample with a value of 63 000 000 copies/mL. This was achieved by diluting the original sample (result = >750 000 copies/mL) 1:10 (NHP) which resulted as >750 000 copies/mL; and was finally diluted 1:100 (NHP) to return a sample value of 630 000 copies/mL, translating to 63 000 000 copies/mL.

Thus, using dilutions, we are satisfied with the linearity of the kit from 400 copies/mL up to at least 70 000 000 copies/mL if we adjust for a coefficient of variation of 10%.

3. **Analytic Sensitivity**

The Analytic Sensitivity was obtained from the Roche Cobas Amplicor HIV – 1 Monitor Applications Handbook.

The studies performed demonstrated that the COBAS AMPLICOR HIV MONITOR Test v1.5 with UltraSensitive specimen processing can detect virion associated HIV-1 RNA in plasma at concentrations As low as 50 RNA copies/ml with a positivity rate greater than 95 % and at concentrations as low as 400 RNA copies/ml with a positivity rate greater than 95% using the Standard specimen preparation procedure, provided that the OD of the selected D-cups is within the specified OD range (0.15 – 2.00).

4. **Analytic Specificity**:

The Specificity was obtained from the Roche Cobas Amplicor HIV – 1 Monitor Applications Handbook.

The clinical specificity of the Cobas Amplicor HIV- 1 MONITOR test, v1.5 was determined by analysis of the anti –HIV – 1 negative blood donors. A total of 507 specimens anticoagulated with either EDTA (267) or ACD (240) were tested by the Standard specimen preparation procedure; 504 specimens anticoagulated with either EDTA (307) or ACD (197) were tested by the Ultrasensitive specimen preparation procedure.All specimens were negative for HIV-1 RNA using the Standard Procedure and 503 of the specimens were negative using the Ultra- sensitive procedure. The one specimen that was positive for HIV-1 RNA with the Ultra- sensitive specimen preparation had an HIV A660 in the neat D-cup of 0.156.When this specimen was retested in duplicate using the Ultra-sensitive procedure, both replicates yielded negative results. Assuming a zero prevalence of HIV – 1 infection in the seronegative blood donors, the clinical specificity of the COBAS AMPLICOR HIV-1 MONITOR Test v1.5 with the Standard processing is 100% and with the Ultra – Sensitive specimen processing is 99.8 %.

The analytical specificity of the COBAS AMPLICOR HIV – 1 MONITOR Test v1.5 was evaluated by adding cultured cells, cultured virus, or purified nucleic acid from the organism and viruses into HIV negative human plasma. Each sample was analyzed using the HIV-1 MONITOR Test v1.5 with Standard specimen processing. None of the non-HIV organisms, viruses or purified nucleic acids tested were positive for HIV-1 RNA. Two of the four HIV – 2 isolates tested (7824 A and 60415K) yielded positive results; however ,no specific claims can be made for the ability of this test to amplify HIV -2 isolates.

5. **Accuracy**

1. 053 20 1097	<400		<400		<400		<400	
2. 053 20 0859	305 000	5.48	103 000	5.01	147 000	5.16	55 600	4.75
3. 053 12 0303	<400		<400		<400		<400	
4 .003 12 1036	<400		<400		<400		<400	
5. 053 12 1111	2 370 000	6.37	763 000	5.88	1 660 000	6.22	1 450 000	6.16
6. 053 12 1125	928 000	5.97	347 000	5.54	447 000	5.65	521 000	5.72
7 .053 12 0366	<400		<400		<400		<400	
8. 053 12 0306	<400		<400		<400		<400	
9. 003 12 2103	445 000	5.65	164 000	5.21	329 000	5.52	292 000	5.47
10. 003 12 1034	2 030 000	6.31	848 000	5.93	2 230 000	6.35	2 610 000	6.42
11. 053 20 1426	2 510	3.4	2 250	3.35	3 640	3.56		
12. 053 12 0328	<400		797	2.9	623	2.8	<400	
13. 053 12 0768	493 000	5.69	341 000	5.53	394 000	5.6	633 000	5.8
14. 053 12 0001	<400		<400		<400		<400	
15. 053 12 0002	<400		<400		<400		<400	
16. 053 12 0247	<400		<400		<400		<400	
17.053 12 1129	795 000	4.9	343 00	4.54	985 000	4.99	51400	4.71
18. 053 20 1096	<400		<400		<400		<400	
19. 053 12 0263	<400		<400		<400		<400	
20. 003 12 2106	1 240	3.09	<400		2 370	3.37	1210	3.08
21. 003 12 2105	27 700	4.44	11200	4.05	46 800	4.67	77800	4.89
22. 003 12 2079	107 000	5.03	85100	4.93	55 900	4.75	77400	4.89
23. 053 12 1115	164 000	5.21	92700	4.97	119 000	5.08	84300	4.93
24. 003 12 2055	15 100	4.18	4700	3.67	24 800	4.39	12 000	4.08
25. 003 12 2110	30 500	4.48	33400	4.52	53 000	4.72	72100	4.86
26. 003 12 2109	9 860	3.99	6310	3.8	15 800	4.2	8530	3.93
27. 003 12 2104	383 000	5.58	279000	5.45	584 000	5.77	648000	5.81
28. 053 20 0530	30 900	4.49	29100	4.46	37 300	4.57	32100	4.51
29. 053 20 0613	<400		<400		<400		<400	
30. 003 12 1033	<400		<400		<400		<400	
31. 053 20 1088	<400		<400		<400		<400	
32. 053 20 0023	<400		<400		<400		<400	
33. 003 12 1045	194 000	5.29	164000	5.21	401 000	5.6	429 000	5.63
34. 003 12 2111	414 000	5.62	244000	5.38	422 000	5.62	376 000	5.78
35.003 12 2088	43100		73400	4.87	111 000	5.05	40300	4.61
36.053 20 0673	<400		<400		<400		<400	
37.053 20 1131	<400		<400		<400		<400	
38.003 12 1063	77800	4.89	136000	5.13	97 300	4.99	37 700	4.58
39.003 12 1060	338000	5.52	289000	5.46	590 000	5.77	333 000	5.52
40.053 12 0004	<400		<400		<400		<400	

Comparison of manual extraction to Ampliprep extraction on viral load data

Since there were two manual and two ampliprep readings per specimen, it was possible to do this report in four different ways:

1) Compare all manual readings against all ampliprep readings
2) Compare the average of the ampliprep readings and the manual readings on each sample

All analyses are done on log transformed viral load values.

Comparison 1: All manual extractions against all ampliprep extractions

Scatterplot of log manual extraction vs log Ampliprep extraction

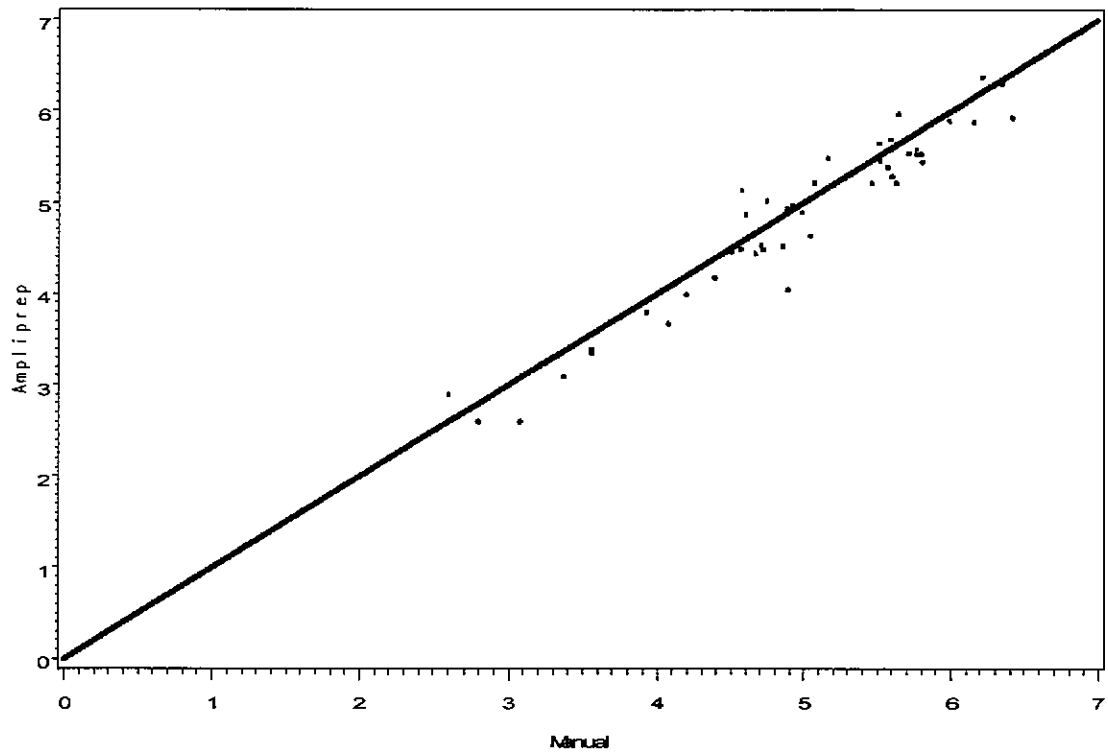

Fig 1: Scatterplot of log viral loads with manual extraction vs Ampliprep, unit is copies per mL

The line of equality gives the point on which all points should lie if the two measurements are exactly equal.

Spearman Correlation coefficient

0.9739 (p-value < 0.0001)

Null hypothesis: The measurements by the two methods are not linearly related.

The null hypothesis is rejected and we can conclude that the viral loads obtained with the two tests are related.

However, showing that the two measurements are related does not prove that they are in agreement.

Plot of the differences between the two tests against their mean

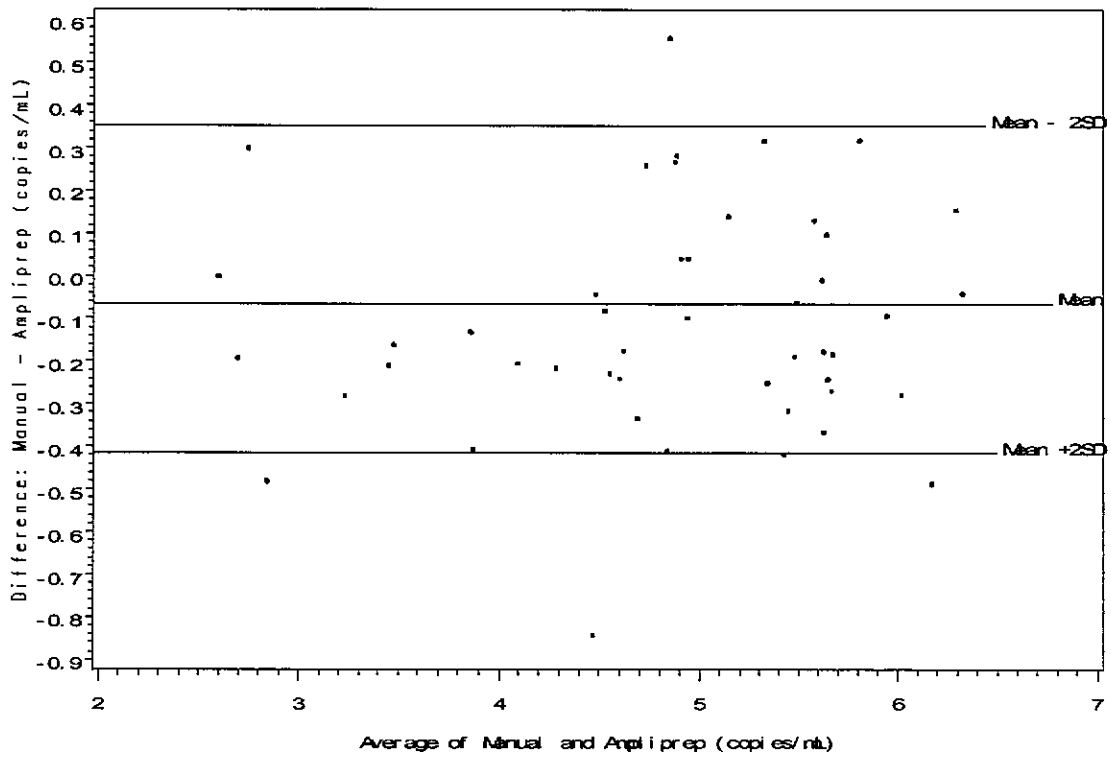

Fig 2: Average of the log viral loads obtained on the two tests versus the difference between the two tests (Manual-Ampliprep). Unit is copies/mL.

Summary of agreement between the two tests

Bias as estimated by the mean difference: -0.065 log copies/mL
Standard deviation of the differences: 0.208

This bias is close to 0, meaning that the viral load obtained with manual preparation and the ampliprep are close to one another.

Bias estimated by the mean difference: 86, meaning that the viral load obtained by the manual extraction is on average 14% lower than the viral load obtained with the ampliprep.

Limit of agreement = (0.33 to 2.24)

For about 95% of the cases the manual extraction result may differ from the ampliprep result by 67% below to 224% above.

Comparison 2: Compare the average of the ampliprep readings and the manual readings on each sample

For these calculations we are comparing the average of the two manual extraction readings to the average of the two ampliprep extraction readings.

Scatterplot of log manual extraction vs log Ampliprep extraction

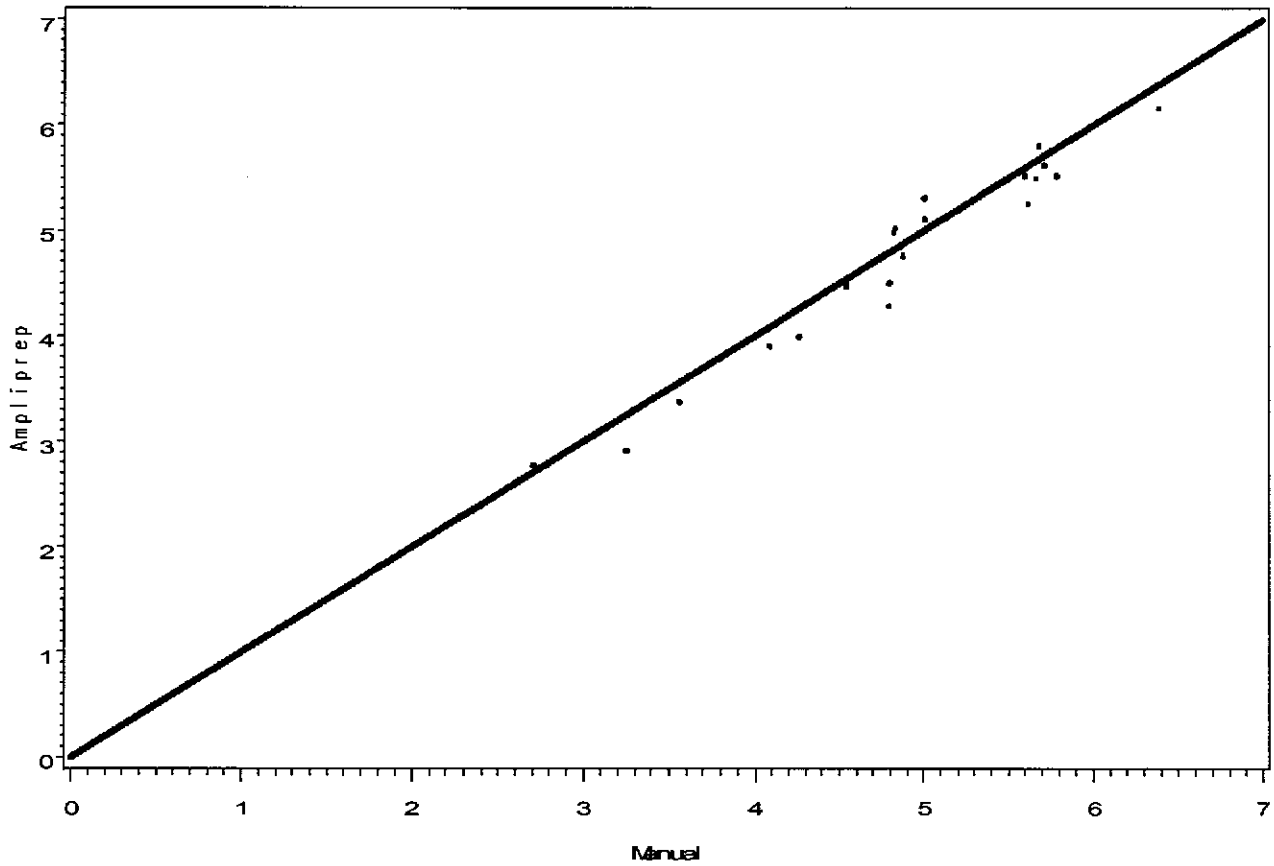

Fig 3: Scatterplot of log viral loads with manual extraction vs Ampliprep, unit is copies per mL

The line of equality gives the point on which all points should lie if the two measurements are exactly equal.

Spearman Correlation coefficient

0.9949 (p-value < 0.0001)
Null hypothesis: The measurements by the two methods are not linearly related.
The null hypothesis is rejected and we can conclude that the viral loads obtained with the two tests are related.
However, showing that the two measurements are related does not prove that they are in agreement.

Plot of the differences between the two tests against their mean

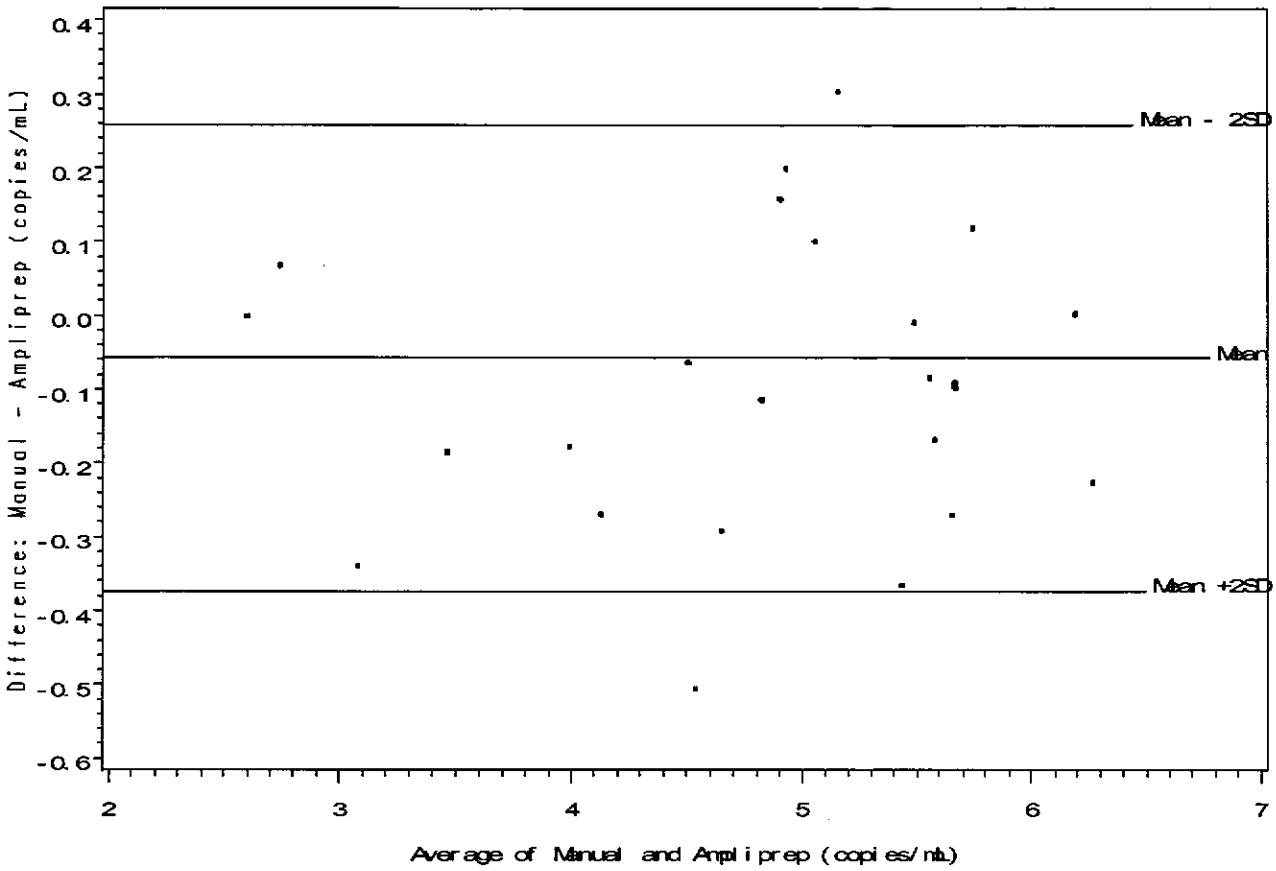

Fig 4: Average of the log viral loads obtained on the two tests versus the difference between the two tests (Manual-Ampliprep). Unit is copies/mL.

Summary of agreement between the two tests

Bias as estimated by the mean difference: -0.057 log copies/mL
Standard deviation of the differences: 0.158

This bias is close to 0, meaning that the viral load obtained with manual preparation and the ampliprep are close to one another.

Bias estimated by the mean difference: 88, meaning that the viral load obtained by the manual extraction is on average 12% lower than the viral load obtained with the ampliprep.

Limit of agreement = (0.43 to 1.81)
For about 95% of the cases the manual extraction result may differ from the ampliprep result by 57% below to 181% above.

5. **Analytical Measurement Range**
   COBAS AMPLICOR HIV – 1 MONITOR Test v1.5 using standard specimen processing is able to detect the highest value being 750 000 copies/ml and the lowest value being <400 copies/ml using undiluted plasma samples.
   COBAS AMPLICOR HIV – 1 MONITOR Test v1.5 using ultraSensitive specimen processing is able to detect the highest value being 750 000 copies/ml and the lowest value being 50 copies/ml using undiluted plasma samples.

6. **Reportable range of patient test results**
   Stated above

7. **Reference ranges or normal values**
   Stated above

8. **Results of Parallel Testing**
   See section on accuracy

DATA ANALYSIS:
The data was analysed using SAS (SAS Institute Inc) version 9.1.

CONCLUSION:
Based on the results obtained and the validation results calculated, it was decided to implement the Ampliprep system as a comparable or superior method of performing extractions. The Ampliprep system is less subjective compared to the manual extraction method..

VERIFICATION AND VALIDATION APPROVAL

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