06	Chlorides	Complies with the limit test for chlorides (50 ppm).	BP
		Complies with the limit test for chlorides (125 ppm)	IP

Apparatus and Chemicals:

250-ml conical flask

Water

2M nitric acid

Silver nitrate

100-ml measuring cylinder

Procedure:

Preparation of Test solution:

To 15 ml of Solution S add 1 ml of 2M nitric acid, pour the mixture as a single addition into 1 ml of silver nitrate solution and allow to stand for 5 minutes protected from light. View transversely against a black background. Any opalescence produced is not more intense than that obtained by treating a mixture of 10 ml of chloride standard solution (75 ppm/100 ppm Cl) and 5 ml of water in the same manner.

Preparation of Standard solution:

Dilute 1 volume of a 0.0824% w/v solution of sodium chloride to 5 volumes with water (to produce 100 ppm), dilute 4 volume of a 0.0824% w/v solution of sodium chloride to 15 volumes with water (to produce 75 ppm) immediately before use.

07	Sulphates	Complies with the limit test for sulphates (50 ppm).	BP
		Complies with the limit test for sulphates (75 ppm).	IP

Apparatus and Chemicals:

250 ml conical flask

Barium chloride

Sulphate standard solution

5M acetic acid

Procedure:

Preparation of Test solution:

Add 1 ml of a 25% 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 Solution S in 15 ml of water 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 preparation.

Preparation of Sulphate standard solution:

Dilute 1 volume of a 0.181% w/v solution of potassium sulphate in distilled water to 20 volumes with the water (50 ppm SO_4), dilute 1 volume of 0.181% w/v solution of potassium sulphate in distilled water to 15 volumes with the water (75 ppm SO_4).

08	Iron	Complies with the limit test for iron (10 ppm)	BP
		Complies with the limit test for iron (20 ppm).	IP

Apparatus and Chemicals:

Separating funnel

Nessler cylinder

Hydrochloric acid

Methyl isobutyl ketone

Citric acid

Mercapto acetic acid

Ammonia

Ammonium iron(III) sulphate

Sulphuric acid

Procedure:

Preparation of Test solution:

In a separating funnel, dissolve 1.0 g in 10 ml of dilute hydrochloric acid. Shake with three quantities, each of 10 ml, of methyl isobutyl ketone, shaking for 3 min each time. To the combined organic layers add 10 ml of water and shake for 3 min. Take 10 ml of the solution 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 colour 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 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 500 ml. Dilute 1 volume to 10 volumes with water (20 ppm Fe). Dilute 1 volume of the solution to 20 volumes with water (10 ppm Fe) immediately before use.

09	Heavy metals	Complies with limit test for heavy metals (20 ppm)	BP
		Not more than 20 ppm.	IP

Apparatus and Chemicals:

Water

Acetate buffer pH 3.5

Thioacetamide reagent

Lead standard solution

Analytical balance

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 0f 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 50 volumes with water (to produce 20 ppm Pb).

10	Arsenic	Complies with the limit test for arsenic (4 ppm).	IP

Apparatus and Chemicals:

250-ml round-bottomed flask

100-ml volumetric flask

3M hydrochloric acid

Water

1M potassium iodide

Zinc AsT

Water bath

Mercuric chloride paper

Procedure:

Preparation of Test solution:

Take 2.5 g of sample and dissolve in 50 ml of water, add 16 ml of brominated hydrochloric acid AsT, and remove the excess of bromine with a few drops of stannous

chloride solution AsT. Add 5 ml of 1M potassium iodide and 10 g of zinc AsT to the solution. 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).

11	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

Apparatus and Chemicals:

Analytical balance

Autotitrator

250-ml conical flask

Anhydrous formic acid

Anhydrous acetic acid

0.1M perchloric acid

Procedure:

Weigh accurately 2.0 g of sample in a 250-ml conical flask, dissolve in about 80 ml of carbon dioxide-free water. Add 0.3 ml of phenolphthalein solution and titrate with 1 M hydrochloric acid. Add 0.3 ml of methyl orange solution and continue the titration with 1 M hydrochloric acid. Calculate the assay of NaOH by given formula.

$$\text{Assay} = \frac{\text{Titer value x Molarity of hydrochloric acid x 40.0 x 100}}{\text{Weight of the sample taken}}$$

$$= \qquad \%$$

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

ANALYTICAL TEST REPORT

Product Information

Material : Sodium hydroxide IP/BP/USP-NF
Item Code : 1000002770
Specification No. : RLS2/QC/RM/SPC018-01
GRN No. :
ATR No. : RLS2/QC/RM/ATR018-01
AR No. :

Sampling Information

Collected By :
Receipt Date :

Analysis Information

S. No.	Parameters	Result	Specification	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	BP
			B. Gives reaction of sodium.	
			A: Gives reaction of sodium salts	IP
			B: pH of a 0.01% w/v solution, not less than 11.0	
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

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 hydroxide
Item Code : 1000002770
Specification No. : RLS2/QC/RM/SPC018-01
GRN No. :
ATR No. : RLS2/QC/RM/ATR018-01
AR No. :

Sampling Information

Collected By :
Receipt Date :

Analysis Information

S. No.	Parameters	Result	Specification	Ref
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
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

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

Analysed By :
Date :

Reviewed By :
Date :

Quality Control

ANALYTICAL TEST REPORT

Product Information [MJ7MS72674]

Material : Sodium hydroxide IP/BP/USP-NF
Item Code : 1000002770
Specification No. : RLS2/QC/RM/SPC018-01
GRN No. : 5806628730
ATR No. : RLS2/QC/RM/ATR018-01
AR No. : AR/RM/08/3924

Sampling Information

Collected By : Sarita Patil
Receipt Date : 25.01.08

Analysis Information

S. No.	Parameters	Result	Specification	Ref
01).	Characters	White crystalline masses, pellets, deliquescent. Solubility Complies	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 pellets, deliquescent.	White, crystalline masses supplied as sticks, pellets or slabs; deliquescent. Readily absorbs carbon dioxide.	IP
02).	Solubility	Complies	Very soluble in water, freely soluble in ethanol (95%).	IP
03)	Identification	Complies	A solution (1 in 25) responds to the tests for Sodium	USP
		Complies	A. pH of the solution is not less than 11.0	BP
		Complies	B. Gives reaction of sodium.	
		Complies	A: Gives reaction of sodium salts	IP
		Complies	B: pH of a 0.01% w/v solution, not less than 11.0	
04).	Insoluble substances and organic matter	Clear & Colourless	Clear, and colorless to slightly colored.	USP
	Appearance of solution	Clear & Colourless	Clear, and colourless.	BP
	Clarity and colour of solution	Clear & Colourless	Clear, and colourless.	IP
05).	Potassium	— NA —	No precipitate is formed	USP
06).	Carbonates	0.49 %	Not more than 2.0 per cent	BP

Result : Sample meets / Does not meets the requirements as per specification.
THIS MATERIAL DOESNOT MEET USP REQUIREMENTS

Analysed By :
Date :

Reviewed By :
Date :

Format No.: RLS2/QC/019/005-00

[REFER ATR FOR B. No. MJ7MS72674]

ANALYTICAL TEST REPORT

Information [MJ7MS72674]

Material : Sodium hydroxide — Item Code : 1000002770

Specification No. : RLS2/QC/RM/SPC018-01 — GRN No. : 5006628730

ATR No. : RLS2/QC/RM/ATR018-01 — AR No. : AR/RM/08/3924

Sampling Information

Collected By : Sarita Patil — Receipt Date : 25.01.08

Analysis Information

S. No.	Parameters	Result	Specification	Ref
07).	Chlorides	Complies	Complies with the limit test for chlorides (50 ppm).	BP
		Complies	Complies with the limit test for chlorides (125 ppm)	IP
08).	Sulphates	Complies	Complies with the limit test for sulphates (50 ppm).	BP
		Complies	Complies with the limit test for sulphates (75 ppm).	IP
09).	Iron	Complies	Complies with the limit test for iron (10 ppm)	BP
		Complies	Complies with the limit test for iron (20 ppm).	IP
10).	Heavy metals		The limit is 0.003%.	USP
		Complies	Complies with limit test for heavy metals (20 ppm)	BP
		Complies	Not more than 20 ppm.	IP
11).	Arsenic	Complies	Complies with the limit test for arsenic (4 ppm).	IP
12).	Assay	97.8 %	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
		97.8 %	Not less than 95.0 per cent and not more than the equivalent of 100.3 per cent of total alkali, calculated as NaOH. Including 3% Na_2CO_3	USP

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

DOESNOT MEET USP REQUIREMENTS

Analysed By :

Date :

Reviewed By

Date :

Division/Dept. : Supply Chain Department (Chemicals)
Telephone 67944319 (D); 0251-2610733

CERTIFICATE OF ANALYSIS

Material : Sodium hydroxide pellets Purified
Material code : 61757390511730
Batch No. : MJ7M572674

RESULTS OF ANALYSIS

TEST	RESULT
Description	[illegible]
Identity	[illegible]
Test solution (10% w/v; water)	[illegible]
Assay (NaOH)	97.2 %
Carbonate (as Na2CO3)	1 %
Chloride (Cl)	0.01 %
Sulfate (SO4)	0.005 %
Heavy metals (as Pb)	[illegible]
Iron (Fe^{3+})	[illegible]

REMARKS : The product COMPLIES with the prescribed standards as per the specification [illegible]

G.M.Dalvi
Quality Assurance

Analysed on : 01.10.2007

This document has been produced electronically and is valid without signature

IN-PROCESS SPECIFICATION
AND
TESTING METHOD

SPECIFICATION – INPROCESS PRODUCT

Material	Albumin Bulk (Inprocess)				
Item Code	None			**Department**	**Quality Control**
Specification No.	RLS1/QC/IP/SPC002	**Revision**	0.0	**Effective Date**	March 31, 2007
Supersedes	None	**Dated**	NA	**Page No.**	Page 1 of 1
Next Review on	Maximum of 2 years from Effective Date				

S. No.	TEST	SPECIFICATION
01	ASSAY (PROTEIN)	None; assay value to be recorded

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION – INPROCESS PRODUCT					
Material	**Albumin Bulk (Final)**				
Item Code	**None**			**Department**	**Quality Control**
Specification No.	RLS1/QC/IP/SPC001	**Revision**	0.0	**Effective Date**	March 31, 2007
Supersedes	None	**Dated**	NA	**Page No.**	Page 1 of 1
Next Review on	Maximum of 2 years from Effective Date				

Storage conditions	At 2 – 8°C
Re-test	After 6 months from the date of manufacture
Parameters for re-test	All (Tests 01 – 05)

S. No.	TEST	SPECIFICATION
01	DESCRIPTION	Clear, slightly viscous liquid, ranging in colour from almost colourless to greenish-yellow or amber
02	pH	Shall be in the range 6.7 – 7.3
03	BIOBURDEN	Shall be < 1cfu / mL
04	BACTERIAL ENDOTOXINS	Shall be < 62.5 EU / mL
05	ASSAY (TOTAL PROTEIN)	• Shall be NLT 10% (for bulk meant for 5% formulation) • Shall be NLT 22% (for bulk meant for 20% formulation)

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

STANDARD TESTING PROCEDURE – INPROCESS PRODUCT					
Material	**Albumin Bulk (Inprocess)**				
Item Code	**None**			**Department**	**Quality Control**
STP No.	RLS1/QC/IP/STP002	**Revision**	0.0	**Effective Date**	March 31, 2007
Supersedes	None	**Dated**	NA	**Page No.**	Page 1 of 2
Next Review	Within a maximum of 2 years from Effective Date				

01	ASSAY (PROTEIN)	None; assay value to be recorded

Procedure: In a set of eppendorf tubes, dilute the protein standard solution (6%) with distilled water to 3%, 1.5% and 0.75%. Prepare each of these dilutions in a single-step from the standard solution (6%). Follow the table below to prepare the different dilutions of the standard.

Standard Conc. (%)	Standard protein solution (µL)	Dist. Water (µL)	Total volume (µL)
6	40	-	40
3	20	20	40
1.5	10	30	40
0.75	10	70	80

Transfer 20 µL from the first three dilutions of the standard to another eppendorf tube labeled with the corresponding concentration of protein. From the tube containing the last dilution (0.75%), transfer 20 µL aliquots to two tubes. Each dilution of the standard is now in duplicate. In a manner similar to the above, dilute the sample eight- and sixteen-fold, and have 20 µL of each of these dilutions in duplicate in eppendorf tubes. Add 1 mL of the Biuret reagent to the two tubes of each dilution of standard and sample. As a blank, use a mixture of 1 mL of Biuret reagent and 20 µL of distilled water in duplicate. Incubate all the tubes (standards, samples and blanks) at 37°C for 15 minutes in an incubator. After the incubation, read the absorbance of the standard and sample

	Prepared by	**Verified by**	**Approved by**
Signature			
Name			
Designation			
Date			

tubes at 546 nm against the blank. Before this, calibrate the cuvettes by adding each of the blanks to one cuvette and zeroing the absorbance at this wavelength. From the absorbance data, plot a calibration curve of Abs. vs. protein concentration. Using the slope and intercept of the line, and the absorbance of the sample dilutions, calculate the protein concentrations of the sample and multiply these by the appropriate dilution factor to arrive at the original protein concentration of the sample (%).

Limits: None; assay value to be recorded

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION – INPROCESS PRODUCT					
Material	**Albumin Bulk (Inprocess)**				
Item Code	**None**			**Department**	**Quality Control**
Specification No.	RLS1/QC/IP/SPC002	**Revision**	0.0	**Effective Date**	March 31, 2007
Supersedes	None	**Dated**	NA	**Page No.**	Page 1 of 1
Next Review on	Maximum of 2 years from Effective Date				

S. No.	TEST	SPECIFICATION
01	ASSAY (PROTEIN)	None; assay value to be recorded

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

FINISHED PRODUCT SPECIFICATION

SPECIFICATION – FINISHED PRODUCT

Material	AlbuRel™ (Human Normal Albumin 20% solution, IP) –50 ml				
Specification No.	SPC/QC/FP/021/01	Effective Date	12 Aug 08	Department	Quality Control
Supersedes	SPC/QC/FP/021/00	Dated	22.01.2008	Page No.	Page 1 of 2

Sampling Quantity : 25 Vials

STP No. : STP/QC/FP/013/01

Reference : IP 2007/ In house

Storage conditions : Store at and below 25° C temperature

	Name	Signature	Date
Prepared By Executive – Quality Control			
Reviewed By Manager – Quality Control			
Reviewed By Executive – Quality Assurance			
Approved By Dy.Manager – Quality Assurance			

SPECIFICATION – FINISHED PRODUCT

Material	AlbuRel™ (Human Normal Albumin 20% solution, IP) –50 ml				
Specification No.	SPC/QC/FP/021/01	Effective Date	12 August	Department	Quality Control
Supersedes	SPC/QC/FP/021/00	Dated	22.01.2008	Page No.	Page 2 of 2

Sr. No.	Test	Specification	Reference
1.	Description	Clear, slightly viscous liquid, ranging in colour from almost colourless to greenish-yellow or amber.	IP
2.	Identification A. Precipitation tests	Monospecific single arc against the Anti human Serum is observed in the given test sample of albumin whereas nonspecific is observed against Anti Horse serum	In house
	B. Electrophoresis	The bands are similar in pattern and placement to the mobility of human albumin test sample when compared with the marker of 66 KDa	In house
3.	Acidity or Alkalinity	Between 6.7 and 7.3	IP
4.	Alkaline Phosphatase	NMT 0.1 U / g protein.	In house
5.	Haem content	Absorbance at 403 nm shall be NMT 0.15	IP
6.	Denatured Protein	NMT 5.0%	IP
7.	Protein Composition	The bands are similar in pattern and placement to the mobility of human albumin test sample when compared with the marker of 66 KDa	In house
8.	Stability	Must comply with the test for stability.	IP
9.	Pyrogens	Must comply with the test for pyrogens.	IP
10.	Sterility	Must comply with the test for sterility.	IP
11.	Abnormal Toxicity	Must comply with the test for abnormal toxicity.	IP
12.	Assay	Between 19% and 21% (95 % and 105 % of the quantity of protein stated on the label.)	IP
13.	Sodium	NMT 160 mmols/L	IP
14.	Potassium	NMT 2 mmols/L	IP
15.	Aluminium	NMT 200 µg/L.	IP
16.	HIV I and II by PCR*	Must not be detected.	In house
17.	HBV by PCR*	Must not be detected.	In house
18.	HCV by PCR*	Must not be detected.	In house
19.	Parvovirus by PCR*	Must not be detected.	In house

FINISHED PRODUCT TESTING METHOD

Standard Testing Procedure of Finished Product

STANDARD TESTING PROCEDURE – FINISHED PRODUCT

Material	Albutrel™ (Human Normal Albumin 20% solution, IP) –50 ml				
STP No.	STP/QC/FP/013/01	Effective Date	12 August	Department	Quality Control
Supersedes	STP/QC/FP/013/00	Dated	22.01.2008	Page No.	Page 1 of 11

Sampling Quantity : 25 Vials

Spec. No. : SPC/QC/FP/021/01

Reference : IP 2007 / In house

Storage conditions : Store at and below 25° C temperature

	Name	Signature	Date
Prepared By Executive – Quality Control			
Reviewed By Manager – Quality Control			
Reviewed By Executive – Quality Assurance			
Approved By Dy.Manager – Quality Assurance			

1. Description

Specification	Clear, slightly viscous liquid, ranging in colour from almost colourless to greenish-yellow or amber.	IP

Check the contents against light. This test is to check that the preparation confirms to its description and is also free from particulate matter.

2. Identification

A. Precipitation tests

Specification	Monospecific single arc against the Anti human Serum is observed in the given test sample of albumin whereas nonspecific is observed against Anti Horse serum	In house

Principle

Ouchterlony is a Immunological test for antigen antibody reactions in which diffusion of soluble antigen and antibody in a gel leads to precipitation of an antigen-antibody complex, visible usually as a whitish band. The precipitin line is more defined when the concentrations of both antigen and antibody are optimum. Even if the concentration of either is more than the other, the precipitin line will show a shift towards the component which is less concentrated.

Reagents

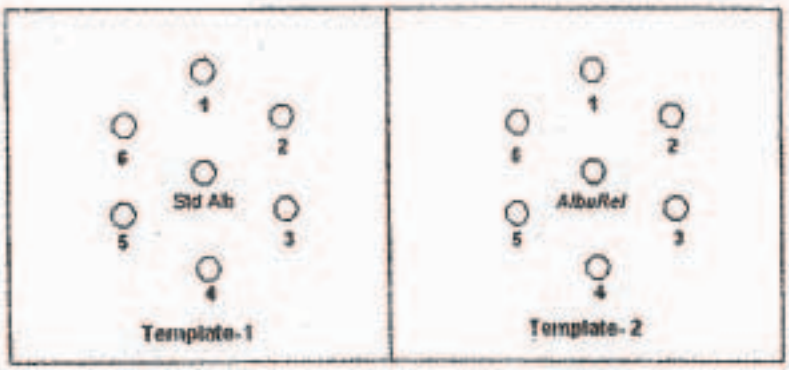
1. Anti human serum albumin developed in Goat (Sigma Aldrich- product code A7544) or equivalent.
2. Anti horse whole serum developed in rabbit (Sigma Aldrich- product code H8890) or equivalent.
3. Human Serum Albumin (Sigma Aldrich- product code A1887) or equivalent.
4. Test vial of *Alburel™*
5. Agarose powder (Promega- cat no.-DV3123) or equivalent.
6. Phosphate Buffer Saline (20mM, pH 7.2-7.4, 0.1% sodium Azide)
7. Coomassie Brilliant Blue R-250 (Himedia Cat No-42660).
8. Methanol
9. Acetic Acid (Glacial) 100% anhydrous.

Equipment

1. Clean Grease free Petri plates/Glass slides (5.0 cm×7.5 cm)
2. Syringe/Cork Borer (0.2 mm inner diameter)
3. Air tight plastic box
4. Clean 10ml plastic pipettes
5. Hot air oven/ 37°C incubator.
6. Cellophane and Whatman paper.

Procedure

1. Prepared 1% Agarose solution in Phosphate Buffer Saline by adding 1 gm of Agarose powered to 100ml of Phosphate Buffer Saline. Digest the Agarose completely by heating.
2. Take clean grease free glass slides as per the requirement and poured 15ml of molten 1% agarose medium on the glass plate in such a manner that it forms a thin, even gel layer on a plate.
3. Do not disturb the plates until gel get solidify. After solidification keep the plates at 2-8°C for 15-20min.
4. With the help of a syringe/cork borer punch a well as per shown in the template 1 and 2. This template has a central well surrounded by six equally spaced wells numbered for identification and record keeping. Number the wells on the bottom of the diffusion plate. The wells must be carefully prepared; nicks at the edges of the well can cause distortions in the precipitin ring.



5. Add 5 µl of 0.5mg/ml Standard Human Serum Albumin to the centered well of template-1 and the test Alburel™ in the template-2 at the same concentration.
6. Add 5 µl of anti Human Serum Albumin to 1, 3 5 wells surrounded by a well containing Standard Albumin and similarly into the wells containing test Alburel.
7. Follow the same above step for Anti horse whole albumin, and Add 5µl of it into the 2, 4, 6 wells respectively in each template.
8. Cover the plates and set them in a moist chamber at room temperature until the precipitin lines appear (18 to 72 hours).
9. The results are best viewed with diffuse back-lighting while holding the opened dish vertically. Precipitin lines vary in shape and intensity depending upon the reactants' concentration and rates of diffusion.
10. Compare the control and test plates. Only the antibodies which are specific for the antigen will form a line of precipitin with the antigen, whereas the non-specific antibodies will not form a line of precipitin.

Drying and Staining of the plates

1. After 18-24hrs of incubation note down the results and keep the ouchterlony plate in DW for 15 min. the gel will get detached from the glass plate.
2. Cut a cellophane paper slightly bigger than the gel size, soak it into the PW for 5Min and wrap it around the glass plate in such a way that there should not be any Air bubble.
3. Carefully put the detached gel on a glass plate having cellophane paper and cover it with the water soaked whatman filter paper.
4. Keep this plate for drying at 37°C for overnight or at 80°cC for 2-3 hrs.
5. After complete drying remove the whatman paper and keep the glass plate in staining solution for 2 hrs.
6. Remove the staining solution and wash the plate for 2 times with the help of PW to remove excess stain.
7. To destain the gel background, keeps the plate in destaining solution until the background gets clear.
8. Now after completion of the detaining rove the destainer and wash the plates 5 times with the help of PW.
9. Drain all the water and put the plate in a vertical position for air drying, for 15-18 hrs.
10. Gel Doc the image and report it.

B. Electrophoresis

Specification	The bands are similar in pattern and placement to the mobility of human albumin test sample when compared with the marker of 66 KDa	In house

Principle

Under the influence of an electrical field, charged particles dissolved or dispersed in an electrolyte solution migrate in the direction of the electrode bearing the opposite polarity. In gel electrophoresis, the movements of the particles are retarded by interactions with the surrounding gel matrix, which acts as a molecular sieve. The opposing interactions of the electrical force and molecular sieving result in differential migration rates according to sizes, shapes and charges of particles. Because of their different physico-chemical properties, different macromolecules of a mixture will migrate at different speeds during electrophoresis and will thus be separated into discrete fractions. Electrophoretic separations can be conducted in systems without support phases (e.g. free solution separation in capillary electrophoresis) and in stabilizing media such as thin-layer plates, films or gels.

SOP for reference: RLS1/QCD015

3. Acidity or Alkalinity

Specification	Between 6.7 and 7.3	IP

Apparatus / Reagents / Glassware

1. Test Sample
2. pH meter (Make Eutech Instrument ID No:RLS/P1/QCA/0044)
3. Normal saline (9 g/L sodium chloride solution)

Preparation of Sample

Dilute the Sample to be examined with normal saline to obtain a solution containing 10 g/L of protein.

Procedure

Rinse electrodes with WFI. Remove water droplets from the tip of electrode using a soft tissue paper. Place the calibrated pH electrode and probe into the 15ml Falcon tube and allow the pH reading to stabilize.

SOP for Reference: RLS1/QCD008

4. Alkaline Phosphatase

Specification	NMT 0.1 U / g protein.	In house

Introduction

Alkaline phosphatase is present in high concentrations in liver, bone, placenta, intestine and certain tumors. Physiologically elevated serum alkaline phosphatase occurs in pregnant women and in growing children. Increased levels of alkaline phosphatase occur in liver diseases, bone diseases (e.g., Rickets, Paget's disease), Hodgkins disease or congestive heart failure. On the other hand, decreased levels occur in hypophosphatasia and malnourished patients.

Principle

In the presence of magnesium ions, alkaline phosphatase converts p-nitrophenyl phosphate to *p*-nitrophenol while releasing phosphate.

$$\text{p-nitrophenyl phosphate} + H_2O \xrightarrow[Mg^{2+}]{ALP} \text{p-nitrophenol} + \text{phosphate}$$

P-nitro phenol absorbs at 405 nm. The increase in absorbance at 405 nm is followed with the help of a continuous monitoring spectrophotometer for duration of 1 min.

Apparatus / Reagents / Glassware

Alkaline Phosphatase Kit

Test sample

UV/Visible Spectrophotometer

Reconstitution of kit

Allow the reagent bottle and the Aqua-4 to attain room temperature. Add the amount of Aqua-4 indicated on the label to the contents of each vial. Swirl to dissolve, do not shake vigorously. After reconstitution, the reagent is stable for 7 days at 2-8°C, and 1 day at room temperature. Discard if the reagent turns turbid or if the absorbance is more than 0.8 at 405 nm against distilled water.

Procedure

In a cuvette, add 1000 µl of working reagent and 20 µl of sample. After a lag time of 60 sec, follow the rise in OD for a further 60 sec.

Calculation

The general formula for converting absorbance to get result in unit/g is:

$$\frac{2713 \times \text{difference}}{\text{Protein content (g/L)}}$$

Where:

Difference: End OD – Start OD

5. Haem content

Specification	Absorbance at 403 nm shall be NMT 0.15	IP

Apparatus / Reagents / Glassware

Test sample

Normal saline

Double-beam UV/Visible Spectrophotometer

Procedure

Dilute the test solution with sufficient saline to produce a solution of 1.0 % w/v of protein. Check the absorbance of the resulting solution at 403 nm using saline as the blank.

6. Denatured Protein

Specification	NMT 5.0%	IP

Introduction

Polymers of albumin are known to raise the dielectric constant of the blood medium, in turn reducing the zeta potential, which leads to agglutination of Rho D antigen in the presence of Anti-Rho D antibody. The overall effect is agglutination of red blood cells due to removal of bound water, change in red cell shape and reduction of ionic strength. Albumin polymers are formed when albumin is prepared by alcohol precipitation and subjected to freeze-drying, heating, etc.

GTP for reference: RLS1/QC/GTP004

7. Protein Composition

Specification	The bands are similar in pattern and placement to the mobility of human albumin test sample when compared with the marker of 66 KDa	In house

Principle and Procedure: Refer 2 (b)

8. Stability

Specification	No change in the appearance of a vial kept at 57°C for 50 hrs. must be observed when compared with to a test sample vial.	IP

Introduction

Since albumin self-polymerizes on heating and since it is manufactured under aseptic process conditions without the addition of any anti microbial agent in the formulation, a stability test is performed.

Procedure

Transfer 10-15ml of test sample to a sterile vial then it is subjected to a temperature of 57°C for 50 hrs followed by visual inspection of vial, no change is observed in the vial as compared with the test sample vial.

9. Pyrogens

Specification	Must comply with the test for Pyrogens.	IP

Procedure: by using Rabbit.

Outsource testing. (2x50ml Vials shall be sent)

10. Sterility

Specification	Must comply with the test for sterility.	IP

Principle

The sterility test is the single most important safety-related test available for release of any biological formulation. The test is spread over 14 days, giving ample time and scope for any microorganism to grow and express itself. The sample is essentially grown in Soybean Casein Digest medium and Fluid Thioglycollate medium. Whereas samples in SCD media are incubated at a temperature of 20°C – 25°C, FTM samples are incubated at 30°C – 35°C. The incubation of the tubes is for duration of 14 days.

Procedure

Perform the test for sterility as per general testing procedure for Sterility taking 20 vials of each batch. Incubated tubes shall be sterile for the duration of full 14 days.

GTP for reference: RLS1/QC/GTP001

11. Abnormal Toxicity

Specification	**Must comply with the test for abnormal toxicity.**	**IP**

Procedure: by Method A and B as per IP 1996 page no A-24.

Outsource testing. (1x50 ml Vial shall be sent)

12. Assay

Specification	**Between 19% and 21% (95 % and 105 % of the quantity of protein stated on the label).**	**IP**

Apparatus / Reagents / Glassware

1. Test sample
2. 0.02 M Hydrochloric acid
3. Calcon Mixture(1:9 $CuSO_4$ and k_2SO_4)
4. 10M NaOH
5. Sat boric acid
6. Mixed Indicator (Methyl red and Methylene blue indicator)

Introduction

Protein content determined by the Kjeldahl method is followed by three steps (Digestion, Distillation, Titration) the protein in sample is digested in concentrated sulphuric acid, in the presence of catalyst potassium Sulphate and copper Sulphate, at 300-400°C. This breaks down the protein to liberate nitrogen in the form of ammonium ions which dissolve in the oxidizing solution and can be later converted to ammonia gas. The ammonia gas formed during distillation is trapped in a saturated solution of boric acid, where it gets converted again to ammonium ions. This is estimated by titrating it against 0.02M hydrochloric acid. The amount of nitrogen gives an estimate of the amount of protein present in the sample.

Procedure

Take 0.2ml of sample to be determined add 1g Calcon mixture and 2ml conc.Sulphuric acid for digestion of sample. after digestion add 20ml WFI to the sample solution and also add 25ml 10M NaOH for distillation during the time of distillation the distillate collected in the collection flask containing 5ml Sat. Boric acid, 5ml WFI and 50µL Mixed indicator, is titrated with 0.02M standarised HCl.Titrate to pink End point and note the burette rdg Calculate the protein content in mg per ml of the preparation being examined, using the expression:

(Blank rdg -spl burette rdg) x estimated Normalityx0.2802x6.25x5 / 0.02

GTP for reference. RLS1/QC/GTP006

13. Sodium *

Specification	NMT 160 mmols/L	IP

Procedure: by Atomic Absorption Spectrophotometry.

Outsource testing.

14. Potassium *

Specification	NMT 2 mmols/L	IP

Procedure: by Atomic Absorption Spectrophotometry

Outsource testing.

15. Aluminium*

Specification	NMT 200 µg/L	IP

Procedure: by Atomic Absorption Spectrophotometry

Outsource testing. (Aliquot of 10 ml shall be sent)

16. HIV I and II by PCR **

Specification	Must not be detected.	In house

This test is performed at DALC, Rabale, Navi Mumbai as per general procedure (RLS2/QC/GTP003 and RLS2/QC/GTP015)

17. HBV by PCR **

Specification	Must not be detected.	In house

This test is performed at DALC, Rabale, and Navi Mumbai as per general procedure (RLS2/QC/GTP006)

18. HCV by PCR **

Specification	Must not be detected.	In house

This test is performed at DALC, Rabale, Navi Mumbai as per general procedure (RLS2/QC/GTP002)

19. Parvovirus by PCR **

Specification	Must not be detected.	In house

This test is performed at DALC, Rabale, Navi Mumbai as per general procedure (RLS2/QC/GTP014)

*One aliquot of 10 ml shall be sent for these tests

**One aliquot of 10 ml shall be sent for these tests

List of General Test Procedures

Sr No	Contents
1	RLS1/QC/GTP004 - Estimation of denatured protein
2	RLS1/QC/GTP001 - Sterility testing
3	RLS2/QC/GTP006 - HBV DNA Detection (Multiplex- Pre X & Pre S)
4	RLS2/QC/GTP003 - HIV-1 Quantitative assay
5	RLS2/QC/GTP015 - HIV-2 Qualitative assay
6	RLS2/QC/GTP002 - HCV RNA Detection by RT PCR
7	RLS2/QC/GTP014 - Parvo Virus DNA PCR
8	Test Procedure for Sodium, Potassium & Aluminum
9	Test Procedure for Pyrogen & Abnormal Toxicity

GENERAL TESTING PROCEDURE					
Description	Estimation of denatured protein			Department	Quality Control
GTP No.	RLS1/QC/GTP004	Revision	0.0	Effective Date	26-04-2007
Supersedes	None	Dated	NA	Page No.	Page 1 of 3
Next Review on	Maximum of 2 years from Effective Date				

PURPOSE

The purpose of this GTP is to describe the procedure followed in the quality control laboratory for estimating denatured protein in samples of plasma products.

SCOPE

This procedure is applicable to the quantitative estimation of denatured protein in samples of blood plasma products originating from the Plasma Products manufacturing group at cGMP 1, Worli, Mumbai

PRINCIPLE

Heating of a protein solution leads to polymerization of a fraction of the proteins. The polymerized protein has a size different from the unpolymerized protein. This size difference permits separation of the polymerized fraction from the unpolymerized fraction using size-exclusion chromatography (SEC). The separation is effected in a column packed with an inert matrix (in the form of porous beads) which allows only molecules of defined sizes to penetrate it. The polymerized fraction of larger-sized molecules does not penetrate the matrix and is eluted out from the column much earlier than the unpolymerized fraction.

MATERIALS

- AKTA PRIME liquid chromatograph system, equipped with a size-exclusion column containing a gel matrix for exclusion of globular proteins of molecular weight 400 – 500 kDa (e.g. Sephadex G 150), UV detector and chart recorder
- Saline solution (0.9% NaCl, autoclaved)
- Mixed phosphate buffer pH 7.0 containing azide V (To 1000 ml of a solution containing 1.6% w/v of disodium hydrogen phosphate and 2.3% w/v of sodium

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GENERAL TESTING PROCEDURE					
Description	Estimation of denatured protein			Department	Quality Control
GTP No.	RLS1/QC/GTP/004	Revision	0.0	Effective Date	26-04-2017
Supersedes	None	Dated	NA	Page No.	Page 2 of 3
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chloride, add sufficient of a solution containing 0.78% w/v of sodium dihydrogen phosphate and 2.3% w/v of sodium chloride (about 280 mL) to produce a pH of 7.0. Dissolve sufficient sodium azide in the resulting solution to give a 0.02% w/v solution).

- Nitrogen-free sulphuric acid
- 7.5% w/v sodium molybdate solution
- Clarified normal human serum
- Water bath at 57°C ± 1°C
- A vial of the sample to be tested

PROCEDURE

Column equilibration and chromatography of Standard

- Equilibrate the column containing the matrix for SEC at 20°C – 25°C with a saline-phosphate solution prepared by mixing 2 volumes of Saline solution and 1 volume of Mixed phosphate buffer (pH 7.0) containing azide.

- Apply to the column 2.5 mL of clarified normal human serum and elute the saline-phosphate solution at a rate of 20 mL per hour.

- Prepare a chromatogram by recording Absorbance of the eluate at 280 nm in relation to its volume. The chromatogram exhibits three well-defined peaks.

- Determine the volume, V, of the eluate from the entry of the sample in the column to the apex of the first peak.

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GENERAL TESTING PROCEDURE					
Description	Estimation of denatured protein			Department	Quality Control
GTP No.	RLS1/QC/GTP004	Revision	0.0	Effective Date	22-04-2017
Supersedes	None	Dated	NA	Page No.	Page 3 of 3
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Chromatography of sample and protein estimation

- Dilute the sample to be tested with the saline-phosphate solution to a concentration of about 6% protein.

- Apply 2.5 mL of the diluted sample to the column and elute under the above conditions, collecting the eluate in 5-mL portions. Three peaks may appear in similar positions to those in the chromatogram obtained from normal human serum, but the relative peak heights may be different.

- To the fraction eluted between 0.85 V and 1.15 V, add for each 10 mL, 0.4 mL of a 7.5% w/v solution of sodium molybdate and 0.4 mL of a mixture of 1 part of nitrogen-free sulphuric acid and 30 parts of water, shake, centrifuge for 5 minutes and estimate the protein as per SOP RLS1/QC0028. The weight of protein in the fraction of the eluate should not be more than 3.5% of the weight of protein in the volume of the sample applied to the column.

Heat treatment of sample, chromatographic separation and protein estimation

- Heat the sample for 50 hours at 56.5°C to 57.5°C and repeat the chromatographic separation and protein estimation in the fraction eluted between 0.85 V and 1.15 V as described above. When expressed as a percentage of the weight of protein in the volume of the sample applied to the column, it should not exceed the percentage obtained in the previous estimation by more than 1.5% w/v.

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GENERAL TESTING PROCEDURE					
Description	Sterility testing				
GTP No.	RLS1/QC/GTP001	**Revision**	04	**Effective Date**	04 June 8
Supersedes	RLS1/QC0001 (Rev) 03	**Dated**	29. June 07	**Page No.**	Page 1 of 12
Next Review		Maximum of 2 years from "Effective Date"			

1. Purpose

The Purpose of this GTP is to lay down the procedure to be followed for Sterility testing.

2. Scope

This Procedure is applicable to the testing of Sterile Finished Products, Raw / Packaging Materials, CBR blood samples & Fresh / Used cell-culture media.

3. Responsibility

The QC Microbiologist is responsible for performing the sterility test and Reporting.

The QC Head is responsible for approving the sterility test result.

The QC Executive and QA Executive are responsible for failure investigation and Reporting.

4. Requirements

4.1 Sterility Testing Filtration units and Samples

4.2 PreSterilized Membrane filters having 0.45 µm porosity with 47mm diameter.

4.3 Isopropyl alcohol (70% v/v)

4.4 Hand disinfectant

4.5 Lint free swipes / Mops

4.6 Sterile scissors & forceps

4.7 Sterile garments with Latex rubber gloves

4.8 Sterile disposable Syringes & Needles

4.9 Presterilized & Pre-incubated Fluid Thioglycollate Medium (FTM) and Soybean Casein Digest medium (SCDM) tubes

4.10 PreSterilized & Pre-incubated Soybean Casein Digest Agar (SCDA) plates

4.11 Sterile & Pre-incubated Peptone water in 500-ml bottles

4.12 Sterile Micropipette and Tips

4.13 Culture suspension of below mentioned Table - I of 10 – 100 Cfu's for Positive control

4.14 Laminar Air Flow

4.15 BOD Chamber - 20°C -25°C & 30°C -35°C

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4.16 Buchner flask (Suction flask)

5. Culture Media used for Sterility Testing

Prepare Culture media for the tests as described below or dehydrated formulations may be used as below.

5.1. Soyabean Casein Digest Medium (HIMEDIA – MM011)

Ingredients	Gms / litre
Pancreatic digest of casein	17.00
Papaic digest of Soyabean meal	3.00
Sodium chloride	5.00
Dibasic potassium phosphate	2.50
Dextrose	2.50
pH	7.1±0.2

Suspend 30.0 grams in 1000 ml Purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into test tubes and sterilize by autoclaving at 15lbs pressure at 121°C for 20minutes.

5.2. Fluid Thioglycollate Medium (HIMEDIA – M009)

Ingredients	Gms / litre
Pancreatic digest of casein	15.00
Yeast extract	5.00
Dextrose	5.50
Sodium chloride	2.50
L-Cystine	0.50
Sodium thioglycollate	0.50
Resazurin sodium	0.001
Agar	0.75
pH	7.1±0.2

GENERAL TESTING PROCEDURE					
Description	Sterility testing				
GTP No.	RLS1/QC/GTP/001	Revision	04	Effective Date	01/10/08
Supersedes	RLS1/QC/0001 (Rev) 03	Dated	29. June 07	Page No.	Page 3 of 12
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Suspend 29.75 grams in 1000 ml Purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense into test tubes and sterilize by autoclaving at 15lbs pressure at 121°C for 20minutes. Cool to 25°C and store in dark place preferably below 25°C.

5.2.1 Note:

Don't use if more than the upper one third of the medium has acquired a pink colour.

6.Suitability Tests

The Media used shall comply with the following tests, to be carried out before or in parallel with the test on the product to be examined.

6.1 Sterility of the Medium

Confirm the sterility of each sterilized medium by incubating portions of the media for 14 days. At the end of 14 days of incubation no turbidity should be observed that which confirms the sterility of medium.

6.2 Growth Promotion tests of Aerobes, Anaerobes and Fungi

Test each lot of Sterilized medium for its growth promoting ability by using 100 cfu /ml of microorganisms as mentioned in Table –I.

Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi.

Media is considered to be suitable if growth is observed in terms of Turbidity within stipulated time.

Table – 1: Strains of the test microorganisms and Medium suitable for use in the growth promotion test.

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GENERAL TESTING PROCEDURE					
Description	Sterility testing				
GTP No.	RLS1/QC/GTP001	Revision	04	Effective Date	06 June 08
Supersedes	RLS1/QC0001 (Rev) 03	Dated	29. June 07	Page No.	Page 4 of 12
Next Review		Minimum of 2 years from "Effective Date"			

Table - 1

Aerobic bacteria	Strain number	Medium
Staphylococcus aureus	ATCC6538, NCTC10788, NCIMB9518	FTM
Pseudomonas aeruginosa	ATCC9027, NCIMB8626	FTM
Bacillus subtilis	ATCC6633, NCIMB8626	SCDM
Anaerobic bacteria		
Clostridium sporogenes	ATCC19404, NCTC532, ATCC11437	FTM
Fungi		
Aspergillus niger	ATCC16404	SCDM
Candida albicans	ATCC10231, NCPF3179	SCDM

7.Diluting and rinsing fluid

7.1 Fluid A

Dissolve 1g of peptic digest of animal tissue in water to make 1 L or Centrifuge to clarify, if necessary, and adjust to a pH of 7.1 ± 0.2. Dispense into containers, and sterilize at 121°C at 15Lbs for 20 minutes.

7.2 Fluid B

To each 1L of Fluid A add 1ml of polysorbate 80, adjust pH to 7.1 ± 0.2, Dispense into containers, and sterilize at 121°C at 15Lbs for 20 minutes. Use this Fluid B for articles containing lecithin or oil.

8.Number of Containers and Sample quantity for Sterility testing.

Use the number of containers for sterility testing as indicated in Table - 2, and sample quantity for testing of sterility as indicated in Table -3.

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GENERAL TESTING PROCEDURE					
Description	Sterility testing				
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Table - 2

Batch Size (containers)	Minimum number of containers to be tested
1. Injectable preparations	
Not more than 100	10% or 4, whichever is greater
More than 100, but not more than 500	10
Batch Size (containers)	Minimum number of containers to be tested
More than 500	2% or 20, whichever is less
2. Bulk solids	
Less than 4	All
4 or more but not more than 50	20% or 4, whichever is greater
More than 50	2% or 10, whichever is greater

Table - 3

Total quantity in each container	Sample quantity for sterility test
For Solids	
Less than 50 mg	Total contents of the container
50 mg or more, but less than 200 mg	Half the contents of a container
200 mg or more	100 mg
For Liquids	
Less than 1 ml	Total contents of the container
1 ml or more, but less than 4 ml	Half the contents of the container
4 ml or more, but less than 20 ml	2 ml
20 ml or more, but less than 100 ml	10% of the contents
100 ml or more	Not less than half the contents unless otherwise specified for the product

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9. Procedure

9.1 Transfer sterilized materials from the autoclave to the trolley and pass through into the pass-box of the sterility-testing laboratory.

9.6 Switch on the HEPA and the UV light.

9.7 Place required numbers of sterile scissors, needles, and forceps in SS box, into the Pass box of the sterility-testing laboratory.

9.8 Place required numbers of sterilized rubber paper wrapped filter assembly units, Suction-flasks (1000 ml) with aluminium foil into the pass box of the sterility testing laboratory.

9.9 Place sterilized lint-free boiler suit with, gloves, head-cover and shoe-covers in a autoclavable bag into the change room.

10. Preparation for Sterility test

10.1 Enter into Sterility testing room by following Entry – Exit procedure as per SOP No: RLS1/QC0011.

10.2 Switch on the LAF Bench.

10.3 Take out the load from the pass box and place it under the cleaned LAF bench.

10.4 Place the SS box at one end of the laminar flow and open its lid.

10.5 Take out a sterilized rubber tube and remove the butter paper from one of its ends.

10.6 Connect the above end to the outlet of the vacuum line or vacuum pump.

10.7 Keep the valve of the vacuum line closed.

10.8 Remove the butter paper at the other end of the rubber tubing and attach the end firmly to the side arm of the suction flask.

10.9 Place the sterility- testing unit on the mouth of the flask and press down firmly.

10.10 The apparatus is now ready for being used for the sterility test.

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GENERAL TESTING PROCEDURE					
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11. Disinfecting and arranging samples under the LAF bench

11.1 Clean the LAF bench with 70% (v/v) isopropyl alcohol.

11.2 Switch on the UV-lights and the blower of the LAF bench to dry the surface.

11.3 Surface-sterilize the containers using a swab/gauze dipped in 70% v/v isopropyl alcohol.

12. Performing sterility test of samples by Membrane Filtration Method

12.1 Open the individual vials with the help of a disinfected SS vial opener.

12.2 In case of lyophilized samples, reconstitute them with sterile WFI.

12.3 The quantity of the sample to be taken from each container for sterility testing is given in the Table II.

12.4 Fit a needle of gauge 17 to a sterile syringe, draw out the contents of a container and add to the filtration unit.

12.5 In case the contents of a container are insufficient for performing the test, pool the contents of two or more containers. Use a separate sterile syringe for drawing out the contents of each container and pool the contents of all the containers into a separate sterile container. Add the pooled sample to the cup of the filtration unit and start the suction.

12.6 After filtering the sample, pass 3 x 100 ml of sterilized peptone water (the whole contents of one flask) through the same filter.

12.7 Turn off the vacuum after the peptone water is filtered, and remove the clamp to separate the two portions of the filtration assembly.

12.8 Lift the Membrane filter disc and cut it into two halves using a pair of sterile forceps and scissors.

12.9 Immerse one half of the filter in FTM broth and the other in SCDM broth.

12.10 Close the tubes with plastic caps.

12.11 Ensure that the filters are completely immersed in the media.

12.12 Have an appropriate positive and negative control along with the experimental units.

12.13 Incubate the tubes of FTM at 30-32°C in an incubator, and the tubes of SCDM at 20-22°C in a BOD incubator for 14 days.

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GENERAL TESTING PROCEDURE					
Description	Sterility testing				
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Note: Carryout Positive Control in Microbiology Room.

13.Sterility testing by Direct Inoculation method

13.1 Transfer the quantity of the preparation to be examined directly into the culture medium with the help of Sterile Micropipette and tips, so that the volume of the product is not more than 10% of the volume of the medium.

13.2 Remove the liquid from the test containers with a sterile pipette or a sterile syringe and needle.

13.3 Aseptically transfer the test sample onto each of the culture medium.

13.4 Incubate the inoculated media for not less than 14 days, at 30° to 35° in the case of FTM and at 20° to 25° in the case of SCDM.

14. Observations and Interpretation Of Results

14.1 Observe the tubes of test sample daily for any signs of growth, and records the observations in the sterility test report.

14.2 When the material being examined renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer suitable portions (each not less than 1 ml) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days.

14.2 Observe the tubes of positive as well as negative control. The negative control tubes Should be clear, and the positive control tubes should exhibit growth within 5 days of incubation.

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GENERAL TESTING PROCEDURE					
Description	Sterility testing				
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Supersedes	RLS1/QC0001 (Rev) 03	Dated	28. June 07	Page No.	Page 9 of 12
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14.3 If no evidence of microbial growth is found, the Product to be examined complies with the test for sterility. If evidence of microbial growth is found, the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for cause unrelated to the product to be examined. The test may be considered invalid only if one or more of the following conditions are fulfilled.

a. The data of the microbiological monitoring of the sterility testing facility shows not complies.
b. A review of the testing procedure used during the test in question reveals a fault.
c. Microbial growth is found in the negative controls.
d. After determination of the identity of the microorganisms isolated from the test, the growth of this species may be ascribed unequivocally to faults with respect to the material and or the technique used in conducting the sterility test procedure.

14.4 If the test is declared to be invalid. It is repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test, the product examined complies with the test for sterility. If the microbial growth is found in the repeat test, the product examined does not comply with the test for sterility.

15. References

15.1 IP 96, Appendix 9.5

15.2 BP 2007, Appendix XVI A

15.3 USP 29

8.0 Annexure(s)

8.1 Annexure – I (Sterility test report format)

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Designation			
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GENERAL TESTING PROCEDURE					
Description	Sterility testing				
GTP No.	RLS1/QC/GTP001	Revision	04	Effective Date	08/Dec/08
Supersedes	RLS1/QC0001 (Rev) 03	Dated	26. June 07	Page No.	Page 10 of 12
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Annexure – I

(Sterility Test Report Format)

STERILITY TEST REPORT

Name of Product				Batch Size	
Batch No.		Mfg. Date		Exp. Date	
Sampled on		Tested on		Reported on	
Report No.		No. Of Containers Tested			
Method	Membrane Filtration / Direct Inoculation				
Quantity used per container / Total Quantity Pooled from different containers					
Details of Media / Filtration membrane (if used):					
Peptone Water	FTM		SCDM	Filtration Membrane	
S. No.	S. No.		S. No.	Make :	
				Type :	
				Pore size & Diameter :	
Prepared on:	Prepared on:		Prepared on:	Lot No.:	
Observations:					

	Daily Observation Record													
Day >>>	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Date >>>														
FT Medium + Sample (30-32°C)														

	Prepared by	Verified by	Approved by
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Designation			
Date			

GENERAL TESTING PROCEDURE					
Description	Sterility testing				
GTP No.	RLS1/QC/STP001	Revision	04	Effective Date	01/10/08
Supersedes	RLS1/QC0001 (Rev) 03	Dated	29. June 07	Page No.	Page 11 of 12
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FT Medium Negative control (20-32°C)														
SCD Medium + Sample (20-25°C)														
SCD Medium Negative control (20-25°C)														
Observed by														
Legend: (P): Growth observed (N): No growth observed														
Result: The above sample complies / does not comply with the test for sterility as per IP/BP/USP/In-house.														
Analysed by: Date:									Checked by: Date:					

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Designation			
Date			

GENERAL TESTING PROCEDURE					
Description	Sterility testing				
GTP No.	RLS1/QC/GTP001	Revision	04	Effective Date	06 June 08
Supersedes	RLS1/QC0001 (Rev) 03	Dated	29. June 07	Page No.	Page 12 of 12
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Declaration		
I have fully understood the above-mentioned procedure, including the associated documentation. I have also undergone training for operation of the same.		
Signature	Name	Date
Certification		
The above-signed persons are proficient in carrying out this procedure.		

Signature		Name		Date	

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Designation			
Date			

GENERAL TESTING PROCEDURE

Description	HBV DNA Detection/Multiplex – Pre X&Pre S			Department	Quality Control
GTP No.	RLS2/QC/GTP005-	Revision	00	Effective Date	Feb 15, 07
Supersedes	None	Dated	NA	Page No.	Page 1 of 7
Next Review on	Maximum of 2 years from effective date				

1 **Purpose**

This GTP describes the detection of Hepatitis B Viral (HBV) DNA in patient's plasma or serum

2. **Scope**

The procedure is an in vitro Qualitative assay of nucleic acid amplification for the detection of HBV DNA in human serum or plasma.

3. **Principle:**

The principle of the test is based on a multiplex in-vitro amplification of Pre X and Pre S region of HBV using viral specific primers in single reaction tube. The process involves 40 cycles of denaturation, amplification, and extension to yield a final product of 358 bp and 553 bp observed on agarose gel electrophoresis. Molecular weight markers are used to size the specific fragment and appropriate positive and negative controls validate the assay.

4. **Requirement (Reagent & Equipment):**

4.1 <u>Reagents for DNA extraction and PCR amplification</u>

- Specimen preparation reagents for extraction of DNA (Qiagen DNA extraction – QIAamp DNA Mini Kit (Refer GTP No RLS2/QC/GTP005-00)

4.2 <u>Amplification reagents</u>

- HBV "Pre X" gene specific primers
- HBVP : 5'GTC CTG CGG AAC TCC TAG C 3'

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GENERAL TESTING PROCEDURE					
Description	HBV DNA Detection(Multiplex – Pre X&Pre S			Department	Quality Control
GTP No.	RLSa/QC/GTP/06	Revision	00	Effective Date	Feb 15, 07
Supersedes	None	Dated	NA	Page No.	Page 2 of
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- HBV2R 5 GTT CAC GGT GGT CTC CAT GCG AGG TGC 3'
- HBV 'Pre S' gene specific primers
- PreS P1 5 GTC CTA GGA ATC CTG AIG 3'
- PreS P2 5' GCG TCA CCA TAT TCT TGG 3'
- 25 mM Magnesium chloride
- 10X PCR Buffer
- Taq DNA Polymerase
- 2mM dNTPs mix
- Positive control DNA
- Negative control DNA
- 100 bp molecular marker
- Orange G loading dye

4.3 Equipments

- 0.2 ml PCR reaction tubes
- 1.5 ml eppendorf tube, 2.0 ml micro centrifuge tubes
- Tube holders'
- Latex gloves
- Graduated cylinder
- Pipettes (5-50, 50-200, 20-200, 0.5-10, 100-1000)µl
- Aerosol barriers pipette Tips for the various pipettes used Micro centrifuge
- Bio-safety laminar hood
- Dry bath (56°C)
- Micro centrifuge
- 2-8°C fridge

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GENERAL TESTING PROCEDURE					
Description	HBV DNA Detection(Multiplex – Pre T&Pre S			Department	Quality Control
GTP No.	RL32/QC/GTP/08	Revision	00	Effective Date	Feb 15, 07
Supersedes	None	Dated	NA	Page No.	Page 3 of 7
Next Review on	Maximum of 2 years from effective date				

- 20°C freezer
- -80°C freezer
- Thermal cycler
- Gel electrophoresis apparatus

4.4 Storage Conditions

- Protease , 100bp molecular weight marker, orange G loading dye: 4°C
- HBV specific primers 25mM Magnesium Chloride, 10X PCR buffer Taq DNA polymerase, 2mM dNTPs mix : -20°C

4.5 Types of specimen requested

- Blood, Plasma, Cord blood to be collected in a sterile vacutainer with anti coagulants EDTA/ACD

4.6 Quantity of Specimen Requested

- 5ml of whole blood or Cord Blood, 2ml of separated plasma

5 **Safety Measures**

- While handling the samples, wear double pair of gloves
- Open the Specimen tubes or containers in the biosafety hood
- Wear proper clothing (Gowning) including lab coat, face mask and gloves or as specified for the area usage
- Open or prepare PCR reagents and master mixes for PCR in laminar air hood only.

6 **Criteria for unacceptable specimen:**

- Heparinised specimens are not suitable for this assay

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Date			

- Reject the Samples if these are not properly identified packed container damaged or Specimen has leaked

7 Procedure

7.1 Introduction

The HBV DNA Detection Test is based on 3 major processes

- DNA extraction.
- Amplification of HBV DNA using HBV specific primers
- Detection of amplified products by agarose gel electrophoresis

Primers HBV F, HBV 2R amplify the Pre X protein gene of HBV and gives an amplified product of 358 bp

Primers PreS P1 and PreS P2 amplify the Pre S gene and give an amplified product of 553 bp.

7.2 Specimen Preparation

7.2.1 Allow all reagents to warm to room temperature (RT=21°C - 24°C). Switch on the UV light of biosafety hood for 20 min, with pipettes tubes and tip boxes kept inside. After 20 min, switch off the UV light and start the airflow of the hood.

7.2.2 Arrange 1.5 ml microcentrifuge tubes in the tube rack. Label each tube. Blood specimens are spun at 2000 rpm for 5 min to separate plasma

7.2.3 With an aerosol barrier tip add 400µl of plasma or whole blood specimen to the appropriate tubes.

7.2.4 Extract DNA from 400 µl plasma or whole blood using QIAGEN DNA extraction protocol (Refer to SOP RLS2/QC)

GENERAL TESTING PROCEDURE

Description	HBV DNA Detection/Multiplex - Pre X&P-e 3			Department	Quality Control
GTP No.	RLS2/QC/GTP/06	Revision	0.0	Effective Date	Feb 15, 07
Supersedes	None	Dated	NA	Page No	Page 5 of 7
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7.2.5 Elute DNA in 50µl of Elution buffer. Extraction DNA is used in the PCR assay. The samples may be stored at -20°C.

7.3 Preparation of Master Mix

7.3.1 Keep pipettes, tubes, tip boxes in the laminar flow hood. Switch on the UV light for 10 min. After 20 min, switch off the UV light and start the airflow.

7.3.2 Identify the required number of samples including positive control, negative control, reaction control, specimens and extra reactions depending upon the total no of reactions. Prepare working Master Mix for the amplification of HBV DNA PCR as follows. The master mix contains the following components (given per reaction):

Stock Solution	1 reaction (µl)
Sterile Milli Q Water	20
10 X PCR Buffer	5.0
2mM dNTPs	5.0
25mM $MgCl_2$	4.0
(10 pmol) HBV P	1.0
(10 pmol) HBV 2R	1.0
(10 pmol) HBV PreS P1	1.5
(10 pmol) HBV PreS P2	1.5
(5U/µl) TaqDNa Polymerase	0.3
Total Volume	40µl

7.3.3 Aliquot 40 µl of master mix in the 0.2ml tubes.

7.3.4 Add 10 µl of sterile milliQ water to the reaction control tube.

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7.3.5 Add 10µl of HBV Negative DNA in the negative control tube.

7.3.6 Add 10µl of the HBV Positive DNA in the positive control tube

7.3.7 Place the tubes into the Thermal Cycler block and close the cover

7.3.8 Program for HBV DNA amplification is as follows:

Initial denaturation	94°C/5 min
Cycles (40 cycles)	94°C/30 sec
	51°C/30 sec
	72°C/30 sec
Final Extension	72°C/5min
Hold	4°C/∞

7.3.9 Start the program. The program runs for approximately 2 hrs.

7.4 Detection of the HBV Amplified Product

7.4.1 Pour a 2% Agarose gel (Refer SOP no. RLS2/QC for Agarose gel electrophoresis.)

7.4.2 Load the 100 bp DNA ladder in the 1st well of the Agarose gel. Load 10 µl of the amplified reaction control, negative control, specimen products and positive control.

7.4.3 Electrophorese the gel for 30 min at 200 volts

7.4.4 Visualize the gel under UV light.

7.4.5 Save the image in HBV folder with unique identification name, take print of the gel for permanent record.

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GENERAL TESTING PROCEDURE

Description	HBV DNA Detection Multiplex – Pro M&Pro S			Department	Quality Control
GTP No.	RnD/QC/GTP/098	Revision	0.0	Effective Date	Feb 15, 07
Supersedes	None	Dated	NA	Page No.	Page 7 of 7
Next Review on	Maximum of 2 years from effective date				

8 **Observation & Conclusion**

8.1 Observation

8.1.1 The Positive control should show the presence of both 553 bp and 358 bp fragment.

8.1.2 The negative control should show an absence of both 553bp and 358 bp fragment.

8.1.3 The presence of both 553bp and 358bp product indicates that the samples are positive for HBV.

8.1.4 The absence of both 553bp and 358bp product indicates that the samples are negative for HBV.

8.2 Method Limitations

8.2.1 The assay detects as few as 1 HBV particle per reaction, equivalent to 1250 viral particles per ml of the sample. The specificity of the assay is 97-99% false negative results may occur if the specimen contains inhibitors to Taq DNA Polymerase such as heparin, SDS, Collegen, fat and protein. Highly lipemic or hemolytic samples may also result in false negative result.

8.3 Cross Reactivity

8.3.1 Due to high level of specificity of the primers and nested primers and the defined size of the product, cross reactivity indicating false positive results are not observed. However as the primer sizes are in the range of 19-27 bp, homology with human genome or other viral/ bacterial genomes may result in shorter or longer amplification.

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GENERAL TESTING PROCEDURE

Description	HIV-1 Quantitative assay			Department	Quality Control
GTP No	RA/02/QC/GTP/003	Revision	0.0	Effective Date	Feb 15, 07
Supersedes	None	Dated	NA	Page No.	Page 1 of 8
Next Review on	Max. 2 years from Effective date				

1. **Purpose**

   This GTP describes the procedure of performing quantitation of Human Immunodeficiency Viral load in patient's plasma in quality control lab.

2. **Scope**

   This procedure covers an in vitro nucleic acid amplification test for the quantitative analysis of determining HIV RNA in human plasma.

3. **Principle**

   The Viral RNA is transcribed to cDNA and serially diluted RNA or cDNA is subjected to PCR. The reaction is visualized on a gel after agarose gel electrophoresis. The intensity of the serially diluted sample is compared to a quantitative ladder, to indicate the ng quantity of PCR products. On multiplication with the dilution factor and calculation factor, the viral quantity in the original sample is estimated.

4. **Material & Equipment:**

   - 25 mM Magnesium chloride
   - 10 X PCR Buffer
   - Taq RNA polymerase
   - 2 mM dNTPs mix
   - Stratascript Reverse Transcriptase Enzyme
   - Reverse Transcriptase Buffer
   - Positive sample for HIV RNA
   - Negative sample for HIV RNA

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GENERAL TESTING PROCEDURE

Description	HIV-1 Quantitative assay			Department	Quality Control
GTP No	RLSD/QC/GTP003	Revision	2.0	Effective Date	Feb 15, 07
Supersedes	None	Dated	NA	Page No	Page 2 of 8
Next Review on	Max. 2 years from Effective date				

- Sterile Milli Q Water
- 100 bp molecular weight marker
- Orange G loading dye
- E-Gel ® Low Range Quantitative DNA ladder
- Reagents for RNA Extraction and PCR amplification
  - Specimen preparation reagents for extraction of RNA (Qiagen RNA extraction - QIAamp® RNA Mini kit (Refer Current GTP Viral RNA Extraction by QIAGEN Kit Method)
  - Amplification reagents, HIV specific primers SK 431: 5'AGT TGG AGG ACA TCA AGC AGC CAT GCA AAT 3' and SK 462: 5' TGC TAT GTC AGT TCC CCT TGG TTC TCT 3'
- Plastic wares:
  - 1.5 ml microcentrifuge tubes
  - 2.0 ml microcentrifuge tubes
  - 0.2 ml PCR reaction tubes
  - Aerosol barrier pipette tips (0.5-10µl, 5-50 µl, 20-200µl, 10 -1000µl)
  - Tubes holder
  - Graduated cylinders
  - Latex gloves
- Equipments
  - Thermal cycler
  - Microcentrifuge
  - Micropipettes (0.5-10µl, 5-50 µl, 20-200µl, 10 -1000µl)
  - 2°C – 8°C Refrigerator
  - -20°C Freezer

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GENERAL TESTING PROCEDURE

Description	HIV 1 Quantitative assay	Department	Quality Control
GTP No.	RL53/QC/GTP/003 Revision	Effective Date	Feb 15,11
Supersedes	None Dated	Page No.	Page 5 of 8
Next Review on	One year from Effective date		

- -80°C Freezer
- Bio-safety Laminar flow hood
- Laminar flow hood
- Gel electrophoresis apparatus

• Storage condition (Reagents)

- 100 bp molecular weight marker & Gel6 Low range Quantitative DNA ladder Orange G loading dye -4°C
- HIV specific primers, 25mM Magnesium Chloride, 10 X PCR buffer Taq DNA Polymerase, 2 mM dNTPs mix Reverse Transcriptase Enzyme, Reverse Transcriptase Buffer: -20°C

5. Safety Precautions

5.1 The followed measures are required for safety

5.2 While handling of sample double pair of gloves is worn.

5.3 Specimen tubes or containers are opened in biosafety hood in a separate sample processing room.

5.4 Proper attire including lab coat, face mask and gloves is worn.

5.5 PCR reagents are opened and master maxes for PCR are made in laminar air flow hood only.

6. Procedure:

6.1 <u>Introduction</u>

6.1.1 HIV RNA Quantitation Test is based on four major processes.

6.1.1.1 RNA extraction from biological specimen.

6.1.1.2 Reverse Transcription of HIV RNA to cDNA using HIV specific primers

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GENERAL TESTING PROCEDURE

Description	HIV-1 Qualitative assay			Department	Quality Control
GTP No	[illegible]	Revision	[illegible]	Effective Date	[illegible]
Supersedes	None	Dated	[illegible]	Page No	Page [illegible] of [illegible]
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6.1.1.3 Amplification of HIV cDNA using HIV specific primers

6.1.1.4 Quantitation of the amplified products by agarose gel electrophoresis.

6.1.2 Primers HIV SK 431 / HIV SK 462 amplify gag region of HIV and gives an amplified product of 142 bp

6.2 <u>Specimen Preparation</u>

6.2.1 Allow all the reagents to warm to room temperature (RT=21°C-24°C) Switch on the UV light of biosafety hood for 20 min, with pipettes, tubes and tip boxes kept inside. After 20 min, switch off the UV light and Start the air flow of the tubes.

6.2.2 Arrange 1.5ml screw cap tubes in the tube rack. Label each specimen preparation tubes.

6.2.3 With an aerosol barrier tip add 140 µl of serum or plasma specimen to the appropriate tubes

6.2.4 Extract RNA by Qiagen Viral RNA Extraction protocol. (Refer Current GTP Viral RNA Extraction by QIAGEN Kit Method.)

6.2.5 Elute the RNA in 50µl of Elution Buffer.

6.3 <u>Reverse Transcriptase (RT) of HIV RNA</u>

6.3.1 Switch on the UV light of the laminar flow hood with pipettes, tubes, tip boxes kept inside. After 20 min switch off UV light and start the flow of air.

6.3.2 Identify the required number of reaction to be made including positive control, negative control, reaction control, specimen and extra reaction according to total no. of reactions to be made

6.3.3 Prepare Master Mix for HIV cDNA synthesis as follows:

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GENERAL TESTING PROCEDURE

Description	HIV-1 Quantitative assay			Department	Quality Control
GTP No	[illegible]	Revision	[illegible]	Effective Date	[illegible]
Supersedes	None	Dated	[illegible]	Page No	[illegible]
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Stock solution	1 reaction (µl)
Sterile milli-H_2O	3.5
10 X RT Buffer	2.5
2 mM dNTPs	2.5
(10pmol) SK 431	1.0
(10pmol) SK 462	1.0
50(U/µl) Reverse Transcriptase	0.5
Total volume	10µl

6.3.4 Aliquot 10µl of master mix in the labeled 0.2 ml tubes.

6.3.5 Add 15µl of milli-Q water in the reaction control tubes, 15µl of negative control RNA in the negative control tubes, 15µl of the specimen RNA sample

6.3.6 Place the tubes in the thermal cycler and close the lid

6.3.7 Run the program for cDNA Synthesis as follows: 42°C / 50 min.

6.4 Preparation of master mix:

6.4.1 Switch on UV light of the laminar flow hood with pipettes, tubes, and tip boxes kept inside. After 20 min switch off the UV light and start the flow of air.

6.4.2 Identify the required number of samples including positive control, negative control, reaction control, specimens and one or more extra reactions, depending upon the total number of reaction.

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GENERAL TESTING PROCEDURE

Description	HIV-1 Quantitative assay			Department	Quality Control
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6.4.3 Prepare working Master Mix for the first round amplification of HIV RNA Detection as follows

Stock solution	1 reaction (µl)
Sterile Milli Q H_2O	17.1
10X PCR Buffer	2.5
25 mM dNTPs	2.5
25 mM MgCl2	10
(5 U / µl) Taq DNA polymerase	0.3
Total Volume	37.5 µl

6.4.4 Aliquot 25µl of PCR Master Mix in each to be containing cDNA

6.4.5 Place the tubes into thermal cycler block and close the lid.

6.4.6 Program for HIV Amplification as follows

- Initial denaturation : 94°C / 5 min
- Cycle program (40 cycles) : 94°C / 30 sec
  55°C / 30 sec
  72°C / 30 sec
- Final extension 72°C / 10 min
- Hold program : 4°C for (∞)

6.4.7 Start the program. The program runs for approximately 2 hr.

6.5 Electrophoresis of HIV Amplified products

6.5.1 2.75 % Agarose gel. Refer to Current GTP for Agarose gel electrophoresis.

6.5.2 Aliquot 2.0µl of orange G on platform.

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GENERAL TESTING PROCEDURE

Description	HIV-1 Quantitative assay	Department	Quality Control
SOP No.	[illegible]	Effective Date	[illegible]
Supersedes	No.	Issue No.	[illegible]
Next Review on	[illegible]		

6.5.1 Load 100 bp DNA Ladder in the first well and E-Gel® Low Range quantitative DNA ladder in the last well of agarose gel. Load one of negative control, reaction control, positive control specific PCR products and the negative control reacted with lysis buffer & sample after mixing with Orange G.

6.5.4 Electrophoresis the gel for 20 min at 20 volts.

6.5.5 Visualize the gel under UV light.

6.5.6 Store the image as .tif image in HIV folder with unique number and take a print of the gel as a record.

7. Observation

7.1 Interpretation and results

7.1.1 Presence of 142 bp products for gag region should be observed in the positive control and the specific band should not be observed in the negative control and reaction control. This validates the assay.

7.1.2 The intensity of the band is compared with the E-Gel® Low Range quantitative DNA ladder to give ng quantity of PCR products and using a multiplication factor and final quantitation in viral copies / ml is done.

7.1.3 Viral copies / ml = ng DNA X Dilution Factor X calculation Factor where calculation factor is 1000

7.2 Method Limitations.

7.2.1 The lower limit in the assay is 1250 copies / ml of sample.

7.2.2 The specificity of the assay is 97-00%.

7.2.3 False negative results may occur if the specimen contains inhibitor(s) to Taq DNA polymerase such as heparin, SDS, collagen, fat and protein.

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GENERAL TESTING PROCEDURE

Description	HIV-1 Quantitative assay			Department	Quality Control
GTP No.	[illegible]	Revision	[illegible]	Effective Date	Feb 15 [illegible]
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7.2.4 Highly lipemic or hemolytic samples may also results in a false negative result

7.2.5 Degradation of viral RNA during transport or otherwise will result in false negative quantitation results.

7.3 Cross Reaction.

7.3.1 Due to a high level of specificity of the primers and the defined size of the product, cross reactivity indicating false positive are not observed.

7.3.2 As the primers size are 24bp, homology to human genome or other viral / bacterial genomes may results in binding consequent amplification.

7.3.3 However, the amplicon size would differ from the specific HIV amplified fragments.

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GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			Department	Quality Control
GTP No.	RLS2/QC/GTP015	Revision	01	Effective Date	29 Aug 08
Supersedes	00	Dated	06 Nov 07	Page No.	1 of 13
Distribution	None				
Next Review on	Max. 2 years from Effective date				

1. **Purpose**

The GTP describes the detection of Human Immunodeficiency Virus-2 in plasma of finished products by PCR in Quality Control laboratory.

2. **Scope**

This procedure is applicable for in-vitro nucleic acid amplification test which is applicable for the detection of HIV-2 RNA in human plasma.

3. **Principle**

The viral RNA is transcribed to cDNA and subjected to PCR. The reaction is visualized on a gel after agarose gel electrophoresis. The final product is of 297 bp of the *prot (protease)* gene of the pol region of the HIV-2 viral genome.

4. **Requirements (Materials & Equipments)**

4.1 Reagents for RNA Extraction and PCR Amplification

4.1.1 Specimen preparation reagents for extraction of RNA (Qiagen RNA extraction - QIAamp® RNA Mini Kit). Refer GTP RLS2/QC/GTP007.

4.1.2 Amplification reagents

4.1.2.1 HIV-2 specific primers

DP20: 5' GAC AGA GGA CTT GCT GCA 3'

DP21: 5' GGC CAT TGT CTC AGT TTT GG 3'

DP28: 5' CAC CTC AAT TCT CTC TTT GGA 3'

DP27 : 5' TAG ATT TAA TGA CAT GCC TAA 3'

4.1.2.2 25 mM Magnesium chloride

4.1.2.3 5 X PCR Buffer

4.1.2.4 Taq DNA Polymerase

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GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			Department	Quality Control
GTP No.	RLS2/QC/GTP015	Revision	01	Effective Date	25 Aug 08
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4.1.2.5 2 mM dNTPs mix

4.1.2.6 High Capacity cDNA Reverse Transcription Kit by Applied Biosystems (Cat No. # 4368813), Novascript or Equivalent Reverse Transcription kit.

4.1.2.7 Positive control for HIV-2 RNA

4.1.2.8 Sterile Milli Q Water

4.1.2.9 100 bp molecular weight marker

4.1.2.10 Agarose

4.1.2.11 10X (Tris borate Ethylene Diamine Tetra Acetic Acid) stock and 1X working TBE Stock

4.1.2.12 6X Orange G loading dye

4.2 Plastic wares and other requirements:

4.2.1 1.5 ml microcentrifuge tubes

4.2.2 2.0 ml microcentrifuge tubes

4.2.3 0.2 ml PCR reaction tubes

4.2.4 Pipettes (0.5-10 µl, 10-100 µl, 20-200 µl, 100-1000 µl)

4.2.5 Aerosol Barrier tips for the appropriate pipette range

4.2.6 Tube holders

4.2.7 Graduated cylinders

4.2.8 Latex gloves (both powdered and powder free)

4.3 Equipments:

4.3.1 Thermal Cycler

4.3.2 Microcentrifuge

4.3.3 Micropipettes (0.5-10 µl, 5-50 µl, 20-200 µl, 100-1000 µl)

4.3.4 2 - 8°C Refrigerator

4.3.5 -20°C Freezer

4.3.6 -80°C Freezer

4.3.7 Laminar air flow hood

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GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			Department	Quality Control
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Distribution	None				
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4.3.8 Gel electrophoresis apparatus

4.3.9 Gel Documentation system

5. Storage Conditions (Reagents)

5.1 100 bp molecular weight marker, 6X Orange G loading dye: Preferably at 2-8°C and -20°C for long term storage.

5.2 HIV-2 specific primers, 25 mM Magnesium Chloride, 5 X PCR buffer, Taq DNA Polymerase, 2 mM dNTPs mix, ABI cDNA Synthesis kit: -20°C

6. Types of Specimen Requested

Plasma collected in sterile glass vials.

7. Quantity of Specimen Requested

1 - 2 ml plasma

8. Criteria for Unacceptable specimen

Samples will be rejected if these are not properly identified, packed, container damaged or specimen has leaked.

9. Precautions

The following measures are taken for safety.

9.1 While handling of samples two pairs of gloves should be worn.

9.2 Specimen tubes or containers should be opened in a separate sample processing area.

9.3 Proper attire including lab coat, face mask and gloves, should be worn during extraction.

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Designation			
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GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			Department	Quality Control
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Distribution	None				
Next Review on	Max. 2 years from Effective date				

9.4 PCR reagents should be opened and master mixes for PCR should be made in laminar air flow hood. Powder free gloves should be worn during this process.

10. Procedure

10.1 Introduction:

HIV 2 RNA Detection Test is based on four major processes:

10.1.1 RNA extraction from biological specimen.

10.1.2 Reverse Transcription of HIV-2 RNA to cDNA.

10.1.3 Amplification of HIV-2 cDNA using HIV2 specific primers.

10.1.4 Detection of the amplified products by agarose gel electrophoresis.

Primers DP20 / DP21 and DP26 / DP27 amplify prot gene of the pol region of HIV-2 and give an amplified first round product of 388 bp and the nested product of 297 bp.

10.2 Specimen Preparation:

10.2.1 Arrange 1.5 ml, 2 ml tubes in the tube rack. Label each specimen preparation tubes.

10.2.2 With an aerosol barrier tip add 280 µl of plasma specimen to the appropriate tubes.

10.2.3 Extract RNA by Qiagen Viral RNA Extraction protocol. (Refer GTP RLS2/QC/GTP007).

10.2.4 Elute the RNA in 50 µl of Elution Buffer.

10.3 Reverse Transcription (RT) of HIV-2 RNA:

10.3.1 Switch on the UV light of the laminar air flow hood, with pipettes, tubes, and tip boxes kept inside. After 20 min, switch off the UV light and start the air flow for 20 min before use.

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Signature			
Name			
Designation			
Date			

GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			Department	Quality Control
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10.3.2 Identify the required number of reaction to be made including positive control, reaction control, specimens and extra reactions according to total number of reactions to be made.

10.3.3 The Master Mix for HIV-2 cDNA Synthesis(using ABI kit reagents) contains following:

Stock Solution	1 reaction (µl)
Sterile MilliQ H_2O	5.25
10 X RT Buffer	2.5
100mM(25X) ABI dNTPs	1.0
10 X (100 ng / µl) ABI Random Hexamers	2.5
(50 U / µl) MultiScribe RT	1.25
Total volume	12.5

10.3.4 Aliquot 12.5 µl of Master mix in all the labeled 0.2 ml tubes.

10.3.5 Add 12.5 µl of Milli Q water in the reaction control tube, 12.5 µl of the specimen RNA in sample tubes and finally 12.5 µl of HIV 2 Positive RNA in the positive control tube.

10.3.6 Place the tubes in the Thermal Cycler and close the lid.

The program for cDNA Synthesis is as follows:

25°C for 10 min

37°C for 2 hr

Hold 4°C (∞)

10.3.7 The Master Mix for HIV 2 cDNA Synthesis(using Stratascript or Novascript or equivalent kit reagents) contains following:

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Stock Solution	1 reaction (µl)
Sterile MilliQ H_2O	3.5
10 X (stratascript/novascript) RT Buffer	2.5
2mM dNTPs	1.0
DP 20 primer	1.0
DP 21 primer	1.0
(50 U / µl) Novascript/Stratascript RT	1.0
Total volume	10

10.3.8 Aliquot 10 µl of Master mix in the labeled 0.2 ml tubes

10.3.9 Add 15 µl of Milli Q water in the reaction control tube, 15 µl of the specimen RNA in 0.2 ml tubes and finally 15 µl of HIV-2 Positive RNA in the positive control tube.

10.3.10 Place the tubes in the Thermal Cycler and close the lid.
The program for cDNA Synthesis is as follows:
42°C for 60 min
4°C Final hold

10.4 Preperation of Master Mix for 1st round of nested PCR :

10.4.1 Identify the required number of reaction to be made including positive control, reaction control, specimens and one or two extra reaction depending upon the total number of reactions to be made. Prepare working Master Mix for the first round amplification of HIV-2 RNA is as follows:

10.4.2 The master mix contains the following components (for RT products synthesized using ABI kit):

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Date			

GENERAL TESTING PROCEDURE					
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Supersedes	00	Dated	06 Nov 07	Page No.	7 of 13
Distribution	Note				
Next Review on	Max. 2 years from Effective date				

Stock Solution	1 reaction (µl)
Sterile MilliQ H_2O	13.2
5 X PCR Buffer	5.0
2 mM dNTPs	2.5
25 mM $MgCl_2$	2.0
(10 pmol) DP20	1.0
(10 pmol) DP21	1.0
(5 U / µl) Taq DNA Polymerase	0.3
Total volume	25.0

10.4.3 The master mix contains the following components (for RT products synthesized using Novascript / Stratascript or equivalent kit) given per reaction:

Stock Solution	1 reaction (µl)
Sterile MilliQ H_2O	15.2
5 X PCR Buffer	5.0
2 mM dNTPs	2.5
25 mM $MgCl_2$	2.0
(5 U / µl) Taq DNA Polymerase	0.3
Total volume	25.0

10.4.4 Aliquot 25 µl of Master Mix in the 0.2 ml tubes with 25µl cDNA.

10.4.5 Place the tubes into the Thermal Cycler block and close the lid.

10.4.6 Run the program for HIV-2 Amplification program as follows:

Initial Denaturation:	94°C / 5 min
Cycles (40 cycles):	94°C / 30 sec
	55°C / 30 sec
	72°C / 30 sec

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Date			

GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			Department	Quality Control
GTP No.	RLS2/QC/GTP015	Revision	01	Effective Date	23 Aug 08
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Distribution	None				
Next Review on	Max. 2 years from Effective date				

Final Extension: 72°C / 5 min

Hold: 4°C (∞)

10.4.7 Start the program. The program runs for approximately 2 hr.

10.5 Preparation of Nested 2nd Round Master Mix

10.5.1 If done on a different day then switch on the UV light of the laminar flow hood, with pipettes, tubes, tip boxes kept inside. After 20 min, switch off the UV light and start the air flow for 20 min before use.

10.5.2 Identify the required number of reactions to be made including positive control, reaction control, specimens and one or two extra reaction depending upon the total number of reaction to be made. Prepare working Nested Master Mix for the Nested 2nd round amplification of HIV-2 RNA.

The master mix contains the following components: (given per reaction)

Stock Solution	1 reaction (µl)
Sterile Milli Q H_2O	14.7
5 X PCR Buffer	5.0
2 mM dNTPs	2.5
25 mM $MgCl_2$	1.5
(10pm / µl) DP26	1.0
(10pm / µl) DP27	1.0
(5 U / µl) Taq DNA Polymerase	0.3
Total volume	26.0

10.5.3 Aliquot 25 µl of PCR Master Mix in 0.2 ml PCR tubes.

10.5.4 Add 2 µl of first round product into respective nested mix tubes.

10.5.5 Place the tubes in the Thermal Cycler block and close the lid.

10.5.6 Run the program for HIV-2 Amplification as follows:

Initial Denaturation: 94°C / 5 min

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Date			

GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			Department	Quality Control
GTP No.	RLS2/QC/GTP015	Revision	01	Effective Date	25 Aug '8
Supersedes	00	Dated	06 Nov 07	Page No.	9 of 13
Distribution	None				
Next Review on	Max. 2 years from Effective date				

Cycles (30 cycles): 94°C / 30 sec
55°C / 30 sec
72°C / 30 sec

Final Extension: 72°C / 5 min

Hold: 4°C (∞)

10.5.7 Start the program. The program runs for approximately 1 and a half hr.

10.6 Electrophoresis of HIV-2 Amplified Product:

10.6.1 2% Agarose gel is used for electrophoresis. Refer to current GTP RLS2/QC/GTP001 for Agarose gel electrophoresis.

10.6.2 Aliquot 2.0 µl of Orange G on Para film. Add the samples to Orange G individually and mix, and load in agarose gel wells. If 5X green PCR buffer is used then load the PCR products directly onto the wells without the use of Orange G.

10.6.3 Load 100 bp DNA Ladder in the first well. Load 10 µl of reaction control, specimen PCR products, and the amplified positive control as last loading sample. Electrophorese the gel for 40 min at 80-120 volts.

10.6.4 Visualize the gel under UV light.

10.6.5 Store the image with proper labeling and date in the respective folder and take a print of the gel as a permanent record.

11. Observation

Interpretation and Results

11.1 Presence of 297 bp nested round product for prot gene of the pol region should be observed in the positive control. The specific band should not be observed in the reaction control. This validates the assay.

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GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			**Department**	**Quality Control**
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Distribution	None				
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11.2 If the amplified product of the sample also shows band as in the positive control then the sample is identified as positive else it is identified as negative for HIV 2 detection assay.

12. Note:

12.1 The Positive Control is obtained from the MME laboratory with its unique identification no. and the viral copies present in it.

12.2 It is first amplified and electrophoresed with an old or previously tested Positive control. If the result or the band obtained is found to be similar then it can be used further as a Positive Control.

12.3 A document of verification is made and reviewed.

13. Abbreviations

13.1	TBE	-	Tris Borate ethylene diamine tetra acetic acid
13.2	PCR	-	Polymerase chain reaction
13.3	DNA	-	Deoxyribo nucleic acid
13.4	RNA	-	Ribonucleic acid
13.5	cDNA	-	Complementary deoxyribo nucleic acid
13.6	RT	-	Reverse transcription
13.7	SDS	-	Sodium dodecyl sulfate
13.8	dNTPs	-	Deoxy nucleoside tri phosphate
13.9	MME	-	Molecular Medicine
13.10	No.	-	Number

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GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			Department	Quality Control
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Distribution	None				
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14. **Method Limitations**

The lower limit in the assay is 1250 copies / ml of sample. The specificity of the assay is 97-99%. False negative results may occur if the specimen contains inhibitor(s) to Taq DNA Polymerase such as heparin, SDS, collagen, fat and protein. Highly lipemic or hemolytic samples may also result in a false negative result. Degradation of viral RNA during transport or otherwise will result in false negative result.

15. **Cross Reaction**

Due to a high level of specificity of the primers and nested primers, and the defined size of the product, cross reactivity indicating false positive results are not observed. As the primer sizes are 18 - 24 bp, homology to human genome or other viral / bacterial genomes may result in binding with consequent non-specific amplification. However, the amplicon size would differ from the specific HIV amplified fragment.

16. **Reference**

16.1 http://www.cdc.gov/ncidod/eid/vol4no4/pieniaze.htmend www.cdc.gov/ncidod/eid/vol4no4/adobe/pieniazek.pdf : Introduction of HIV-2 and Multiple HIV-1 Subtypes to Lebanon

16.2 http://www.aidsonline.com/pt/re/aids/pdfhandler.00002030-200402200-00016.pdf;jsessionid=LzrQm2HWRd91jTcW79g8VT4tGJp3LgfY1kRGSy2KnLnQ9QTBW16!2126095447!181195829!8091!-1: AIDS official journal of International AIDS society.

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Designation			
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GENERAL TESTING PROCEDURE					
Description	HIV-2 Qualitative assay			Department	Quality Control
GTP No.	RLS2/QC/GTP015	Revision	01	Effective Date	23.8.11
Supersedes	00	Dated	01 Nov 07	Page No.	12 of 13
Distribution	None				
Next Review on	Max. 2 years from Effective date				

17. Annexure

Annexure No.	Details/Title of Annexure	Format No. (Current Version)
QC/GTP015/A01	Format HIV 2 PCR Assay Report	RLS2/QC/GTP015/F01
QC/GTP015/A02	Format for HIV 2 PCR Assay Report using ABI kit	RLS2/QC/GTP015/F02

Revision History	
Changes	1. 5X and 0.5X TBE is no longer mentioned in the GTP. 2. Correction made in the gene amplified i.e. prot gene of pol region instead of gag region (typographical error) and the required reference is also included for it in point no.16. 3. "Separate sample processing room" has been modified as "separate sample processing area". 4. The use of an alternate 5X green buffer which eludes the use of Orange G is mentioned. 5. An additional Note about verification of the Positive Control used is added. 6. Details about the Title and Format no. of Annexures are properly mentioned. 7. The following changes are made in the annexure QC/GTP015/A01 and QC/GTP015/A02: 7.1 Done by, Reviewed By with Date and Page Number has been added in all the pages. 7.2 Equipment codes required for all the equipments have been included in the annexure

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GENERAL TESTING PROCEDURE					
Description	HSV-2 Qualitative assay			Department	Quality Control
GTP No.	RLS2/QC/GTP015	Revision	01	Effective Date	25 Aug 08
Supersedes	00	Dated	06 Nov 07	Page No.	13 of 13
Distribution	None				
Next Review on	Max. 2 years from Effective date				

Reason for Changes	1. 5X and 0.5X TBE are no longer used. 2. Separate sample processing room is always not available hence a separate sample processing area is mentioned. 3. Use of 5X green buffer consumes less time for loading. 4. Positive Controls obtained from MME laboratory needs to be verified before being used as Controls. 5. All kinds of typographical errors are corrected. 6. The GTP and the Annexures are updated to improve the document.

	Prepared by	Verified by	Approved by
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Name			
Designation			
Date			

Format HIV 2 PCR ASSAY Report

[redacted]

1. **Sample Details:**
   Total Number of Samples:

Nature of Sample:	Accession No.	Nature of Sample:	Accession No.

Done By:
Date:

Reviewed By:
Date:

Format HIV 2 PCR ASSAY Report

[redacted]

Sample Details Continued:

Nature of Sample:	Accession No.	Nature of Sample:	Accession No.

Done By:
Date:

Reviewed By:
Date:

Format HIV 2 PCR ASSAY Report

2. Reagents used :

Reagent Details	Kit Name	Lot Number	Exp. Date
Strata/Nova script RT			
Strata/Nova script Buffer			
_____ PCR Buffer			
_____dNTPs			
_____Mgcl2			
DP 20 Primers			
DP 21 Primers			
DP 26 Primers			
DP 27 Primers			
_____Taq Polymerase			

3. Preparation of Master Mix for RT round (1 reaction):

Sterile Milli Q (µl)	_____ Strata script RT buffer(µl)	_____ dNTPs	_____ DP 20	_____ DP 21	_____ Strata script RT(µl)

4. Total No. of reactions:

Vol. of Sterile Milli Q Added	Vol. of _____ Strata script buffer Added	Vol .of _____ dNTPs	Vol .of _____ DP 20	Vol. of _____ DP 21	Vol. of Strata script RT

Done By:
Date:

Reviewed By:
Date:

Format HIV 2 PCR ASSAY Report

[redacted]

5. **Sample Preparation**

MASTER MIX(µl)	Sample(µl)	Vol Of master mix added:	Vol of Sample added

6. **RT PCR Run**
Equipment Code:

THERMOCYCLER
Start Time:
End Time:

7. **Preparation of Master Mix for HIV PCR 2nd round :(1 reaction)**

Sterile Milli Q (µl)	(_____)PCR buffer (µl)	(_____)dNTPs (µl)	(_____)Mgcl2(µl)	(_____)Taq (µl)

8. **Total No. of Reactions:**

Vol of Sterile Milli Q Added(µl)	Vol of (_____)PCR buffer Added(µl)	Vol of (_____) dNTPs Added(µl)	Vol of (_____)Mgcl2 Added(µl)	Vol of (_____) Taq Added(µl)

Done By:
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Reviewed By:
Date:

Format HIV 2 PCR ASSAY Report

[redacted]

9. **Sample preparation for HIV 2 PCR 1st round:**
Total No. of Samples:

MASTER MIX (µl)	PCR product of RT round (µl)	Vol of master mix added :
		Vol of PCR product RT added:

10. **PCR RUN HIV 2 1st round**
Equipment Code:

PCR RUN (Thermocycler)	
Start Time:	
End Time:	

11. **Preparation of Master Mix for HIV 2 Nested PCR (1 reaction):**

Sterile Milli Q (µl)	PCR buffer (µl)	dNTPs (µl)	MgsI2 (µl)	DP 26 (µl)	DP 27 (µl)	Taq Polymerase (µl)

Done By:
Data:

Reviewed By:
Date:

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[redacted]

12. **Total No. of reactions:**

Vol of Sterile Milli Q Added	Vol of (_____) PCR buffer Added	Vol of (_____) dNTPs Added	Vol of (_____)Mgcl2 Added	Vol of (_____) DP 26 Added	Vol of (_____) DP 27 Added	Vol of (_____) Taq Added

13. **Sample preparation for Nested PCR**

MASTER MIX (µl)	PCR product of 1st round (µl)	Vol. Of MASTER MIX Added :
		Vol. of PCR product round 1 added :

14. **PCR RUN HIV 2 nested round:**
Equipment Code:

PCR RUN (Thermocycler)	
Start Time:	
End Time:	

Done By:
Date:

Reviewed By:
Date:

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[redacted]

15. **Electrophoresis:**

Quantity of _____ Agarose prepared:
Lot No. of Agarose:
Quantity of EtBr added:
TBE Stock Prepared on:
TBE working Buffer prepared on: _____
TBE Buffer autoclaved: YES / NO

16. **Sample preparation:**

Sample(µl)	_____Loading Dye(if used))µl	Volume of sample/ Ladder/PC/NC:
		Volume of Loading Dye (if used) :

17. **Agarose Gel Electrophoresis:**
Equipment Code:

Percentage Gel	Conc. of TBE	Voltage	Time	AGE Start Time:
				AGE End Time:

18. **Gel Documentation:**
Equipment Code:
Source of Illumination:

Done By :

Date :

Reviewed By :

Date :

Quality Control	Annexure : QC/GTP015/A02 Page 1 of 7

Format for HIV 2 PCR Assay Report using ABI kit

HIV 2 PCR Assay Report using ABI kit

1. **Sample Details:**
   Total Number of Samples:

Nature of Sample:	Accession No.	Nature of Sample:	Accession No.

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Date:

Reviewed By:
Date:

Format for HIV 2 PCR Assay Report using ABI kit

[redacted]

Sample Details Continued:

Nature of Sample:	Accession No.	Nature of Sample:	Accession No.

Done By:
Date:

Reviewed By:
Date:

Format for HIV 2 PCR Assay Report using ABI kit

[redacted]

2. Reagents:

Reagent Details	Kit Name	Lot Number	Exp. Date
ABI Reagents			
PCR Buffer			
dNTPs			
Mgcl2			
DP 20 Primers			
DP 21 Primers			
DP 26 Primers			
DP 27 Primers			
Taq Polymerase			

3. Preparation of Master Mix for RT round (1 reaction):

Sterile Milli Q (µl)	10X ABI RT buffer(µl)	25X ABI dNTPs	10X ABI Hexamers	50U/ µl ABI RT(µl)
5.25	2.5	1.0	2.5	1.25

4. Total No. of reactions:

Sterile Milli Q (µl)	ABI RT buffer(µl)	ABI dNTPs	ABI hexamers	ABI RT(µl)

5. Sample Preparation

MASTER MIX(µl)	Sample(µl)	Vol Of MASTER MIX Added:
12.5	12.5	Vol of Sample added :

Done By:
Date:

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Date:

Format for HIV 2 PCR Assay Report using ABI kit

6. **RT PCR Run Equipment Code:**

Thermocycler
Start Time:
End Time:

7. **Preparation of Master Mix for HIV PCR 2ND round :(1 reaction)**

Sterile Milli Q (µl)	(5X)PCR buffer (µl)	(2mM)dNTPs (µl)	(25mM) Mgcl2 (µl)	(10pM) DP 20(µl)	(10pM) DP 21(µl)	(5U/ µl)Taq (µl)
13.2	5.0	2.5	2.0	1.0	1.0	0.3

8. **Total No. of Reactions:**

Sterile Milli Q (µl)	( )PCR buffer (µl)	( )dNTPs (µl)	( ) Mgcl2 (µl)	( ) DP 20(µl)	( ) DP 21(µl)	( )Taq (µl)

9. **Sample preparation for HIV 2 PCR 1st round:**
Total No. of Samples:

MASTER MIX (µl)	PCR product of RT round (µl)	Vol Of MASTER MIX Added :
25	25	Vol of PCR product RT added:

Done By:
Date:

Reviewed By:
Date:

Format for HIV 2 PCR Assay Report using ABI kit

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10. PCR RUN HIV 2 1st round
Equipment Code:

PCR RUN (Thermocycler)	
Start Time:	
End Time:	

11. Preparation of Master Mix for HIV 2 Nested PCR (1 reaction):

Sterile MilliQ (µl)	(5X) PCR buffer (µl)	(2mM) dNTPs (µl)	(25mM) Mgcl2 (µl)	(10pM) DP 26 (µl)	(10pM) DP 27 (µl)	(5u/ µl) Taq Polymerase (µl)
14.7	5.0	2.5	1.5	1.0	1.0	0.3

12. Total No. of reactions:

Vol. of Sterile Milli Q added	Vol of (_____) PCR buffer Added	Vol of (_____) dNTPs Added	Vol of (_____) Mgcl2 Added	Vol of (_____) DP 26 Added	Vol of (_____) DP 27 Added	Vol of (_____) Taq Added

Done By:
Date:

Reviewed By:
Date:

Format for HIV 2 PCR Assay Report using ABI kit

[redacted]

13. **Sample preparation for Nested PCR**

MASTER MIX (µl)	PCR product of 1st round (µl)	Vol. Of master mix added :
25	2	Vol. of PCR product round 1 added :

14. **PCR RUN HIV 2 nested round:**
Equipment Code:

PCR RUN (Thermocycler)	
Start Time:	
End Time:	

15. **Electrophoresis:**
Quantity of 2% agarose prepared:

Lot No. of Agarose:

Quantity of EtBr added:

TBE Stock Prepared on:

TBE working Buffer prepared on:

Done By:
Date:

Reviewed By:
Date:

Format for HIV 2 PCR Assay Report using ABI kit

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16. Sample preparation:

Sample(µl)	Loading Dye(If used) µl	Volume of sample/ Ladder/PC/NC:
		Volume of Loading Dye (if used) :

17. Agarose Gel Electrophoresis
Equipment Code:

Percentage Gel	Conc. of TBE	Voltage	Time	AGE Start Time:
				AGE End Time:

18. Gel Documentation
Equipment Code:
Source of Illumination:

Done By:
Date :

Reviewed By:
Date:

GENERAL TESTING PROCEDURE

Description	HCV RNA Detection by RT-PCR			Department	Quality Control
GTP No	RLS/QC/GTP/002	Revision	3.0	Effective Date	[illegible]
Supersedes	None	Dated	N/A	Page No.	Page 1 of 16
Distribution	None				
Next Review on	Max. 2 years from Effective date				

1. **Purpose**

This GTP describes the procedure of performing detection of Hepatitis C Viral RNA for 5' UTR region, Core and Envelop region in patient's plasma or serum in quality control lab.

2. **Scope**

This procedure covers a qualitative in vitro nucleic acid amplification test for the detection of HCV RNA in human serum or plasma

3. **Principle**

The principle of the test is based on reverse transcription of HCV RNA to cDNA and followed by in-vitro amplification of a specific region of the virus using viral specific primers followed by nested PCR in a region internal to the initial PCR. The process involved 30-40 cycles of denaturation, amplification and extension to yield a final product of 244 bp and 474 bp for 5'UTR region, Core and envelop region respectively observed on agarose gel electrophoresis. Molecular weight markers are used to size the specific fragment and appropriate positive and negative controls validate the assay

4. **Material And Methods**

- <u>Reagent for RNA Extraction and PCR Amplification</u>

➢ Specimen preparation reagents for extraction for RNA (Qiagen RNA extraction – QIAamp RNA Mini Kit (Refer Current GTP, Viral RNA Extraction from QIAGEN kit method)

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GENERAL TESTING PROCEDURE					
Description	HCV RNA Detection by RT-PCR			Department	Quality Control
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Supersedes	None	Dated	NA	Page No.	Page 2 of 15
Distribution	None				
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- Amplification Reagents
  - HCV Primers For 5'UTR, HCV-S 5' GCA GAA AGC GTC TAG CCA TGG CGT 3' and HCV-AS 5' CTC GCA AGC ACC CTA TCA GGC AGT 3'
  - HCV Primers for Core and Envelop region, OCE1F 5' GCA ACA GGG AAC CCT GGT TGC TC 3' ; OCE1R 5' CGT AGG GGA CCA GTT CAT CAT CAT 3' ; ICE1F 5' AAC CTT CCT GGT TGC TCT TTC TCT AT 3' and ICE1R 5' GTT CAT CAT CAT ATC CCA TGC CAT 3'
- 25mM Magnesium chloride
- 10 X PCR Buffer
- Taq Polymerase
- 2 mM dNTPs mix
- Reverse transcriptase Buffer
- Reverse transcriptase Enzyme
- ABI Reverse Transcriptase Kit
- 100 bp Ladder
- Positive sample for HCV RNA
- Negative sample for HCV RNA
- Sterile Milli Q water
- Orange G Dye
- Plastic Wares:
- 1.5 ml micro centrifuge tubes
- 2 ml micro centrifuge tubes
- 0.2ml PCR reaction tubes
- Aerosol barrier pipette tips (0.5-10 µl, 5-50 µl, 20-200 µl, 100-1000µl)

	Prepared by	Verified by	Approved by
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Name			
Designation			
Date			

GENERAL TESTING PROCEDURE					
Description	HCV RNA Detection by RT-PCR			Department	Quality Control
GTP No	RLS2/QC/GTP002	Revision	0.0	Effective Date	Feb '15
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Next Review on	Max. 2 years from Effective date				

- Tubes holders
- Graduated cylinders
- Latex gloves
- Equipment
- Thermal Cycler
- Micro centrifuge
- Micropipettes (0.5-10 µl, 5-50 µl, 20-200 µl, 100-1000µl)
- 2°C - 8°C Refrigerator
- -20°C freezer
- -80°C Freezer
- Bio-safety Laminar flow hood
- Laminar flow hood
- Gel electrophoresis apparatus

5. Safety Precautions

5.1 The following measures are taken for safety.

5.2 While handling of samples doubles pair of gloves is worn

5.3 Specimen tubes or containers are opened in safety hood in a separate samples processing room

5.4 Proper attire including lab coat, face mask and gloves is worn

5.5 PCR reagents are opened and master mixes for PCR made in laminar air flow hood only.

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GENERAL TESTING PROCEDURE					
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6. **Procedure**

6.1 Introduction

6.1.1 The HCV Detection Test is based on four major processes

6.1.2 RNA extraction from biological specimen

6.1.3 Reverse Transcriptase of HCV RNA to cDNA

6.1.4 Amplification of HCV cDNA using HCV specific primers in 5' untranslated region (5' UTR) and Core-Envelop (CE) region. CE PCR products are then put for 2nd round amplification

6.1.5 Detection of the amplified products by agarose gel electrophoresis. Primers HCV S / HCV AS amplified the 5'UTR of HCV gives an amplified product of 244 bp fragments.

6.1.6 Primers specific for CE amplify Core and envelop region of HCV and give an amplified products of 474 bp

6.2 Specimen Preparation:

6.2.1 Allow all the reagents to warm to room temperature (RT = 21°C - 24°C) Switch on the UV light of safety hood for 20 min. with pipettes, tubes and tip boxes kept inside. After 20 min, switch off the UV light and start the airflow of the hood.

6.2.2 Arrange 1.5 ml screw cap tubes rack. Label each specimen preparation tubes

6.2.3 With an aerosol barrier tip add 140 µl of serum or plasma specimen to the appropriate tubes.

6.2.4 Extract RNA by Qiagen viral RNA Extraction protocol (Refer Current GTP, Viral RNA Extraction from QIAGEN kit method)

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6.2.5 Elute the RNA in 50µl of elution Buffer.

6.3 Reverse Transcriptase (RT) of HCV RNA:

6.3.1 Switch on the UV light of the laminar flow hood with pipettes, tubes tip boxes kept inside. After 20 min switch off the UV light and start the flow of air.

6.3.2 Identify the required number of reactions including positive control, negative control, reaction control, and specimens and extra reaction depending upon the total number of reactions prepare the RT Master Mix using ABI KIT as follows:

Stock solution	1reaction (µl)
Sterile Milli Q water	5.25µl
10 X RT Reaction Buffer	2.5µl
(10ng / µl) random Primers	2.5µl
25 X ABI dNTPs	1.0µl
(50 U / µl) MultiScribe RT	1.25µl

6.3.3 Aliquot 12.5 µl of master mix in to the labeled 0.2 ml PCR tubes.

6.3.4 Add 12.5µl of sterile Milli Q Water for reaction control, 12.5µl of negative control (HCV Negative RNA), and 12.5µl of specimen RNA samples to the respective tubes and finally add 12.5µl of positive samples for HCV RNA.

6.3.5 Put the tubes in the thermal cycler. The program for cDNA synthesis is as follows

- Incubation at 25°C for 10 min

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- Incubation at 37°c for 2 hr
- Incubation at 4°c (∞)

6.4 Preperation of Master Mix

6.4.1 Switch on UV light of the laminar flow hood with pipettes, tubes and tip boxes kept inside. After 20 min switch off the UV light and start the flow of air.

6.4.2 Identify the required number of samples including positive control, negative control, reaction control, specimens and extra reactions depending upon the total number of reaction. Prepare working Master Mix for the amplification of HCV RNA Detection as follows:

6.4.3 The Master Mix contains the following components: (given per reaction)

Stock solution	1 reaction (µl)
Sterile Milli Q H2O	26.7
10 X PCR Buffer	2.5
25 mM dNTPs	2.5
25 mM MgCl2	1.0
(10 pmole) HCV S	1.0
(10 p mole) HCV AS	1.0
(5 U / µl) Taq DNA polymerase	0.3
Total Volume	37.5 µl

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6.4.4 Aliquot 37.5µl of Master Mix in the labeled 0.2 ml tubes and add 12.5µl DNA to the respective tubes

6.4.5 Place the tubes into thermal cycler block and close the lid

6.4.6 Program for HCV RT PCR for 5'UTR is as follows

- Initial denaturation: 94°C / 5 min
- Cycle program (40 cycles): 94°C / 30 sec
  55°C / 30 sec
  72°C / 30 sec
- Final extension: 72°C / 10 min
- Hold program: 4°C for (∞)

6.4.7 Start the program. The program runs for approximately 2 hr

6.5 <u>Preparation of Master Mix for 1st Round for Core and Envelop Region:</u>

6.5.1 Switch on the UV light of the laminar flow hood with pipettes, tubes, and tip boxes kept inside. After 20 min switch off the UV light and start the flow of air.

6.5.2 Identify the required number of reaction including positive control, negative control, reaction control, specimens and extra reactions depending upon the total number of reaction. Prepare working master Mix for the 1st Round for Core and Envelop Region

Stock solution	1 reaction (µl)
Sterile Milli Q H2O	28.7
10 X PCR Buffer	2.5
2 mM dNTPs	2.5

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25mM MgCl2	1.5
(10pmol) OCE1F	1.0
(10pmol) OCE1R	1.0
(5 U / µl) Taq DNA polymerase	0.3
Total volume	37.5µl

6.5.3 Aliquot 37.5 µl of master mix in the labeled 0.2 tubes and add 12.5µl cDNA to the respective tubes

6.5.4 Place the tubes into the thermal cycler block and close the lid

6.5.5 The program for HCV RT PCR 1st round PCR is as follows:

- Initial denaturation: 94°C / 5 min
- Cycle program (40 cycles): 94°C / 30 sec
  59°C / 30 sec
  72°C / 30 sec
- Final extension: 72°C / 10 min
- Hold program: 4°C for (∞)

6.5.6 Start the program. The program runs for approximately for 1 hr 15 min

6.6 <u>Preparation of Master Mix – Nested PCR for Core and Envelop Region</u>

6.6.1 Switch on the UV light on the Laminar flow hood with pipettes, tubes, and tip boxes kept inside. After 20 min switch off the UV light and start the flow of air.

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Description	HCV RNA Detection by RT-PCR			Departments	Quality Control
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6.5.2 Identify the required number of samples including positive control, negative control, reaction control and specimens. Prepare working master mix for Core –Envelope. Nested mix

Stock solution(µl)	1 reaction
Sterile Milli Q H2O	14.2
10 X PCR Buffer	2.5
2 mM dNTPs	2.5
25mM MgCl2	1.5
(10pmol) OCE1F	1.0
(10pmol) OCE1R	1.0
(5 U / µl) Taq DNA polymerase	0.3

6.6.3 Aliquot 23µl of master mix in the labeled 0.2 ml tubes

6.6.4 Add 2µl of the first round product into the respective nested mix tubes starting with the reaction control, negative cole, Test specimen and finally add the positive control

6.6.5 Place the tubes into the thermal cycler block and close the lid

6.6.6 The program for HCV CE NESTED PCR is as follows.

- Initial denaturation: 94°C / 5 min
- Cycle (30 cycles): 94°C / 30 sec
  57°C / 30 sec
  72°C / 30 sec
- Final extension: 72°C / 10 min

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GENERAL TESTING PROCEDURE					
Description	HCV RNA Detection by RT-PCR			Department	Quality Control
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- Hold program 4 C for (∞)

6.6.7 Start the program. The program runs for approximately 1hr 15 min

6.7 cDNA synthesis by stratascript enzyme

6.7.1 Switch on the UV light on the Laminar flow hood with pipettes, tubes, tip boxes kept inside. After 20 min switch off the UV light and start the flow of air.

6.7.2 Identify the required number of reaction including positive control, negative control, reaction control, specimens and extra reactions depending upon the total number of reaction

Prepare master mix for HCV cDNA synthesis as follows

Stock solution	1reaction (µl)
Sterile Milli Q H2O	5.0
10 X RT Buffer	2.5
2mMdNTPs	1.0
(10 pmol) HCV AS	1.0
(50 U /µ l) Reverse Transcriptase	0.5

6.7.3 Aliquot 10 µl of master mix in the labeled 0.2 ml tubes

6.7.4 Add 15 µl of the first round product into the respective nested mix tubes starting with the reaction control, negative control, test specimen and finally add the positive control

6.7.5 Place the tubes into the thermal cycler block and close the lid

6.7.6 Run the program for cDNA synthesis as follows:

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25°C for 10 min

37°C for 2hr

Hold 4°C (∞)

6.7.7 Prepare working master mix for the amplification of HCV 5' UTR region as follows

Stock solution	1reaction (µl)
Sterile Milli Q H2O	29.7
10 X PCR Buffer	2.5
2 mM dNTPs	2.5
25mM MgCl2	1.5
(10pmol) HCV 5'	1.0
(5 U / µl) Taq DNA polymerase	0.3
Total volume	37.5µl

6.7.8 Aliquot 37.5 µl of master mix in the labeled 0.2 ml tubes

6.7.9 Add 12.5µl cDNA to the respective tubes

6.7.10 Run the program as follows:

- Initial denaturation 94°C / 5 min
- Cycle (30 cycles) 94°C / 30 sec
  55°C / 30 sec
  72°C / 30 sec
- Final extension 72°C / 10 min

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- Hold program 4°C for (∞)

6.6 cDNA synthesis for CE Region

6.6.1 Switch on the UV light on the Laminar flow hood with pipettes, tubes, and tip boxes kept inside. After 20 min switch off the UV light and start the flow of air

6.6.2 Identify the required number of reaction including positive control, negative control, reaction control, specimens and extra reactions depending upon the total number of reaction.

Prepare master mix for HCV cDNA synthesis as follows:

Stock solution	1 reaction (µl)
Sterile Milli Q H2O	10.0
10 X RT Buffer	2.5
2mM dNTPs	1.0
(10 pmol) OCE 1R	1.0
(50 U /µ l)Reverse Transcriptase	0.5

6.6.3 Aliquot 15 µl of master mix in the labeled 0.2 ml tubes

6.6.4 Add 10 µl of the first round product into the respective nested mix tubes starting with the reaction control, negative core, test specimen and finally add the positive control

6.6.5 Place the tubes into the thermal cycler block and close the lid

6.6.6 Run the program for cDNA synthesis as follows:

45°C for 50 min

Hold 4°C (∞)

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Prepare master mix for 1st Round for Core and Envelop Region as follows

Stock solution	1 reaction (µl)
Sterile Milli Q H2O	27.2
10 X PCR Buffer	2.5
2mM dNTPs	2.5
25 mM Mgcl2	1.5
(10 pmol) OCE 1R	1.0
(50 U /µ l)Reverse Transcriptase	0.3
Total volume	35.0µl

6.8.7 Aliquot 37.5 µl of master mix in the labeled 0.2 tubes and add 15µl cDNA to the respective tubes.

6.8.8 Place the tubes into the thermal cycler block and close the lid

6.8.9 The program for CE 1st round PCR is as follows

- Initial denaturation 94°C / 5 min
- Cycle program (40 cycles) 94°C / 30 sec
  50°C / 30 sec
  72°C / 30 sec
- Final extension 72°C / 05 min
- Hold program 4°C for (∞)

6.8.10 Prepare the master mix- nested PCR for Core and Envelop Region.

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Master Mix – Core and Envelope Region – Nested PCR

Stock solution	1 reaction (µl)
Sterile Milli Q H_2O	14.2
10X PCR Buffer	2.5
2 mM dNTPs	2.5
25mM $MgCl_2$	1.5
(10pmol) OCE1F	1.0
(10pmol) OCE1R	1.0
(5 U / µl) Taq DNA polymerase	0.3
Total volume	23µl

6.8.11 Aliquot 23 µl of master mix in the labeled 0.2 tubes.

6.8.12 Add 2µl of the first round product to the respective tubes.

6.8.13 Place the tubes into the thermal cycler block and close the lid.

6.8.14 The program for 1st round PCR is as follows:

- Initial denaturation: 94°C / 5 min
- Cycle program (40 cycles) 94°C / 30 sec
  59°C / 30 sec
  72°C / 30 sec
- Final extension: 72°C / 05 min
- Hold program 4°C for (∞)

6.8.15 Start the program.

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6.9 Detection of the HCV amplified product (5'UTR and CE):

6.9.1 Pour a 2% agarose gel. Refer to SOP no. MDG 0014 for Agarose gel electrophoresis. The product for 5' UTR and CE are loaded on separate gels.

6.9.2 Load 5µl of 100 bp DNA Ladder in the first well of the agarose gel. Load 10µl of the reaction control in the 3rd well, negative control in the last well, specimen products in between wells. The amplified positive control is loaded last in 2nd well as a last loading specimen.

6.9.3 Electrophoreses the gel under UV light.

6.9.4 Visualize the gel under UV light.

6.9.5 Store the image as .tif image in a HCV folder with unique identification number and take a print of the gel as a record.

7. Observation

7.1 Interpretation and Result

7.1.1 Presence of 244 products for 5'UTR 474 bp product in core and envelope region should be observed in the positive control and specific band should not be observed in negative control and / or reaction control. This validates the assay.

7.1.2 The samples showing presence of both 244 bp and 471 bp is considered as positive for HCV RNA and the samples not showing presence of both 244 bp and 474 bp is considered as negative HCV RNA.

7.2 Method Limitation

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2.1 The assay detects as few as one HCV RNA particle per reaction, equivalent to 100 viral particles per ml of the sample.

2.2 The specificity of the assay is 97-99%.

2.3 False negative may occur if the specimen contains inhibitor(s) to Taq DNA polymerase such as heparin, SDS, collagen, fat and protein

2.4 Highly lipemic or hemolytic samples may also result in a false negative result.

3 <u>Cross Reaction.</u>

3.1 Due to a high level of specificity of the primers and nested primers and the defined size of the product, cross reactivity indicating false positive results are not observed.

3.2 However , as the primer size are in the range of 24-26 bp, harmology with human genomes or viral / bacterial genomes may result in shorter or longer amplicons.

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1. **Purpose**

This GTP describes the procedure for the detection of Parvo Virus DNA in plasma of finished products.

2. **Scope**

The procedure is applicable for in vitro qualitative nucleic acid amplification assay for the detection of Parvo Virus DNA in finished products at the Quality Control laboratory.

3. **Principle**

The principle of the test is based on an in vitro amplification of a specific region of the virus DNA using viral specific primers. The process involves 40 cycles of denaturation, amplification and extension to yield a final product of 591 bp observed on agarose gel electrophoresis. Molecular weight markers are used to size the specific fragment and appropriate positive and reaction controls are used to validate the assay.

4. **Requirements (Materials & Equipments)**

4.1 Reagents for DNA Extraction

Refer GTP No. RLS2/QC/GTP005 for reagents required for DNA extraction by QIAamp DNA Mini Kit

4.2 Amplification reagents:

4.2.1 Parvo Virus B19 specific primers

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NeP3: 5' AAT GAA AAC TTT CCA TTT AAT GA 3'

NeP4: 5' TCC TGA ACT GGT CCC GGG GAT GGG 3'

4.2.2 25 mM Magnesium Chloride

4.2.3 5 X PCR Buffer

4.2.4 Taq DNA Polymerase

4.2.5 2 mM dNTPs mix

4.2.6 Sterile Milli Q water

4.2.7 Positive control –Parvo DNA maintained in a plasmid vector

4.2.8 100 bp molecular weight marker

4.2.9 Orange G loading dye

4.2.10 Agarose

4.3 Other requirements

4.3.1 1.5 ml micro-centrifuge tubes

4.3.2 2.0 ml micro-centrifuge tubes

4.3.3 0.2 ml PCR reaction tubes

4.3.4 Aerosol barrier pipette tips (0.5-10 µl, 10-50 µl, 10-200 µl, 100-1000 µl)

4.3.5 Tube holders

4.3.6 Graduated cylinders

4.3.7 Latex gloves

4.3.8 Agarose

4.3.9 10X TBE stock

4.3.10 100bp Molecular weight marker

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4.4 Equipments:

4.4.1 Thermal Cycler

4.4.2 Microcentrifuge

4.4.3 Dry bath (56°C)

4.4.4 Micropipettes (0.5-10 µl, 10-100µl, 20-200 µl, 100-1000 µl)

4.4.5 2°C - 8°C Refrigerator

4.4.6 -20°C Freezer

4.4.7 Laminar flow hood

4.4.8 Gel electrophoresis apparatus

4.4.9 Gel Documentation System

5. Storage Conditions (Reagents)

5.1 Protease, Orange G loading dye: 4°C

5.2 Parvo Virus specific primers, 25 mM Magnesium Chloride, 5 X PCR buffer, Taq DNA Polymerase, 2 mM dNTPs mix: 100 bp molecular weight marker : -20°C

6. Types of Specimen Requested

Plasma of finished products in sterile glass vial

7. Quantity of Specimen Requested

1 - 2 ml of plasma

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8. Criteria for unacceptable specimen

Samples will be rejected if these are not properly identified, packed, container is damaged or specimen has leaked.

9. Safety Precautions

The following measures are taken for safety.

9.1 While handling of samples double pair of gloves should be worn.

9.2 Specimen tubes or containers should be opened in a separate sample processing area.

9.3 Proper attire including lab coat, face mask and gloves should be worn.

9.4 PCR reagents are opened and master mixes for PCR should be made in laminar air flow hood only.

10. Procedure

10.1 <u>Introduction</u>

The Parvo Virus DNA detection is based on three major processes:

10.1.1 DNA extraction from biological specimen

10.1.2 Specific amplification of the desired gene by NeP3 and NeP4 primers to yield 591bp PCR product

10.1.3 Detection of the amplified products by agarose gel electrophoresis.

10.2 Primers NeP3 / NeP4 amplify non structural NS1 gene and give an amplimer of 591 bp.

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10.3 DNA Extraction

10.3.1 Allow all the reagents to warm to room temperature (RT = 21°C - 24°C). Arrange 1.5 ml. microcentrifuge tubes in the tube rack. Label each tube.

10.3.2 With an aerosol barrier tip add 200 µl of plasma to the appropriate tubes.

10.3.3 Extract DNA from 200 µl of sample using Qiagen DNA extraction Protocol (Refer to GTP No. RLS2/QC/GTP005).

10.3.4 Elute the DNA in 100 µl of elution buffer. Extracted DNA is used in the PCR assay.

10.4 Preparation of Master Mix:

10.4.1 Switch on the UV light of the laminar flow hood with pipettes, tubes, and tip boxes kept inside. After 20 min switch off the UV light and start the flow of air.

10.4.2 Identify the required number of samples including positive control, reaction control, specimens and extra reactions depending upon the total no. of reactions. Prepare working Master Mix for the amplification of Parvo Virus DNA PCR as follows:

Table 1:

Stock Solution	1reaction (µl)
Sterile Milli Q H_2O	19.7
5 X PCR Buffer	10.0
2 mM dNTPs	5.0
25 mM $MgCl_2$	3.0
(10 pmol) NeP3	1.0
(10 pmol) NeP4	1.0
(5 U / µl) Taq DNA Polymerase	0.3
Total volume	40.0

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10.4.3 Aliquot 40 µl of Master Mix into labeled 0.2 ml tubes.

10.4.4 Add 10 µl of the sterile Milli Q water to the reaction control tube.

10.4.5 Add 10 µl of the specimen DNA to the respective sample tubes.

10.4.6 Add 10 µl of Parvo Virus positive DNA to the positive control tube.

10.4.7 Place the tubes in the Thermal Cycler block and close the lid.

10.4.8 The program for Parvo Virus DNA amplification is as follows:

Initial Denaturation:	94°C / 5 min
Cycles (40 cycles):	94°C / 30 sec
	58°C / 30 sec
	72°C / 30 sec
Final Extension:	72°C / 7 min
Hold:	4°C (∞)

10.4.9 Start the program. The program runs for approximately 2 hr.

10.5 Detection of the Parvo Virus B19 Amplified Product:

10.5.1 Pour a 2.0% Agarose gel. Refer to GTP no. RLS2/QC/GTP001 for Agarose gel electrophoresis.

10.5.2 Electrophorese the gel for 40 min at 80-120 volts.

10.5.3 Visualize the gel under UV light in a Gel Documentation system.

10.5.4 Save the image in its respective folder with a unique identification name.

10.5.5 Take a print of the gel for permanent record.

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11. **Observation(Interpretation & Results)**

11.1 The Positive control should show presence of 591 bp fragment. The reaction control should show absence of 591 bp fragment.

11.2 Presence of 591 bp product indicates that the samples are positive for Parvo Virus B19 DNA.

11.3 Absence of 591 bp product indicates that the samples are negative for Parvo Virus B19 DNA.

12. **Note:**

12.1 The Positive Control (cloned Parvo DNA maintained in a plasmid vector instead of viral DNA) is obtained from the MME laboratory

12.2 It is first amplified and electrophoresed with an old or previously tested Positive control. If the result or the band obtained is found to be similar then it can be used further as a Positive Control.

12.3 A document of verification is made and reviewed.

13. **Abbreviation**

TBE	-	Tris borate ethylene diamine tetra acetic acid
PCR	-	Polymerase Chain Reaction
dNTPs	-	Deoxy Nucleside Tri Phosphates
UV	-	Ultra Violet
bp	-	Base Pairs
DNA	-	Deoxyribonucleic acid

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

GENERAL TESTING PROCEDURE					
Description	Parvo Virus DNA PCR			Department	Quality Control
GTP No.	RLS2/QC/GTP014	Revision	01	Effective Date	23 Aug 08
Supersedes	00	Dated	22 Nov 07	Page No.	8 of 9
Distribution	None				
Next Review on	Max. 2 years from Effective date				

RT - Room Temperature
No. - Number

14. Method Limitations

The assay detects as few as 100 Parvo Virus particles per reaction, equivalent to1250 viral particles per ml of the sample.

15. Cross Reaction

Due to a high level of specificity of the primers and the defined size of the product, cross reactivity indicating false positive results are not observed. However, as the primer sizes are in the range of 20 bp, homology with human genome or other viral / bacterial genomes may result in shorter or longer amplicons.

16. Annexure

Annexure No.	Details/Title of Annexure	Format No. (Current Version)
QC/GTP014/A01	Format for Parvo PCR assay report	RLS2/QC/GTP014/F01

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

GENERAL TESTING PROCEDURE					
Description	Parvo Virus DNA PCR			Department	Quality Control
GTP No.	RLS2/QC/GTP014	Revision	01	Effective Date	25 Aug 08
Supersedes	00	Dated	22 Nov 07	Page No.	9 of 9
Distribution	None				
Next Review on	Max. 2 years from Effective date				

Revision History	
Changes	1. Under Safety Precautions the term "sample processing area" is mentioned instead of "sample processing room". 2. All types of typographical errors corrected. 3. An additional Note about verification of the Positive Control used is added. 4. The following changes are made in the annexure QC/GTP014/A01 4.1 Done by, Reviewed By with Date and Page Number has been added in all the pages. 4.2 Equipment codes required for all the equipments have been included in the annexure.
Reason for Changes	1. Sample processing room is not mentioned as it is not available all the time instead sample processing area is mentioned. 2. Positive Controls obtained from MME laboratory needs to be verified before being used as Controls. 3. Changes made to update the GTP and Annexure for the improvement of document.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

Format for PARVO PCR Assay Report

PARVO PCR Assay Report

1. Sample Details:
Total Number of Samples:

Nature of Sample:	Accession No.	Nature of Sample	Accession No.	Nature of sample	Accession No.

Done By:
Date:

Reviewed By:
Date:

Format for PARVO PCR Assay Report

PARVO PCR Assay Report

2. Reagents:

Reagent Details	Kit Name	Lot Number	Exp. Date
(_____) PCR Buffer			
(_____)dNTPs			
(_____)Mgcl2			
NeP3			
NeP4			
(_____)Taq polymerase			

3.Preparation of MASTER MIX (1 reaction):

Sterile MilliQ (µl)	(_____) PCR Buffer (µl)	(_____) dNTPs (µl)	(_____) Mgcl2 (µl)	(_____) NeP3 (µl)	(_____) NeP4 (µl)	(_____) Taq (IU/µl)

Done By:
Date:

Reviewed By:
Date:

Quality Control	Annexure : QC/GTP014/A01 Page 3 of 4

Format for PARVO PCR Assay Report

PARVO PCR Assay Report

4. Total No. of reactions:

Vol.of Sterile Milli-Q Added (µl)	Vol of (____) PCR Buffer Added (µl)	Vol of (____) dNTPs Added (µl)	Vol of (____) Mgcl2 Added (µl)	Vol of (____) NeP3 Added (µl)	Vol of (____) NeP4 Added (µl)	Vol of (____) Taq Added (µl)

5. Sample preparation:

MASTER MIX (µl)	Sample/NC/PC/RC (µl)	Vol. of Master Mix added:
		Vol. of sample added:

6. PCR RUN

Equipment Code :

PCR RUN (Thermocycler)	
Start Time:	
End Time:	

Done By:
Date:

Reviewed By:
Date:

Format for PARVO PCR Assay Report

PARVO PCR Assay Report

7. Electrophoresis:

Lot No. of Agarose: **Quantity of 2% agarose prepared:**

(_____)TBE Stock Prepared on: **Quantity of EtBr added:**
(_____)TBE working Buffer prepared on: **(_____) TBE Buffer autoclaved: YES/NO**

8. Sample preparation:

Sample(µl)	Loading Dye(if used) µl	Volume of sample/ Ladder/PC/NC:
		Volume of Loading Dye (if used) :

9. Agarose Gel Electrophoresis Equipment Code:

Percentage Gel	Conc. of TBE	Voltage	Time	AGE Start Time:
				AGE End Time:

10.Gel Documentation System Equipment code:
Source of Illumination:

Done By:
Date:

Reviewed By:
Date:

PACKING MATERIAL SPECIFICATIONS

SPECIFICATION - PACKAGING MATERIAL					
Material	**Seal F/O 33mm/50ml Hook Type Cream Colour**				
Item Code	**1200009062**			**Department**	**Quality Control**
Specification No.	RLS2/QC/PM/SPC079	**Revision**	00	**Effective Date**	Jun 08, '07
Supersedes	None	**Dated**	NA	**Page No.**	Page 1 of 2
Next Review on	Maximum of 2 years from effective date				

Storage conditions	Store at Room Temperature in designated area.
Re-testing period	One year

S. No.	Test	Specifications
01	Description	33 mm Flip top hook type aluminium Seal with Cream Colour plain Polypropylene disc.
02	Total Height	15.00 mm ± 0.30 mm
03	Aluminium Thickness	0.21 mm ± 0.02 mm
04	Base Diameter	33.00 mm ± 0.30 mm
05	Disc Height	4.25 mm ± 0.30 mm
06	Disc Diameter	36.25 mm ± 0.30 mm
07	Embossing	Plain Disc
08	Total Weight	3.00 g ± 0.02 g
09	Formation	Seal is hook type. Hook designed such that it is hidden inside the polypropylene disc. Flip the disc to reach the circular hook. Hold the outer circular part of the hook and pull it up for hanging the bottle.
10	Packing	Seal packed in Double Polybag, in turn in shipper. Material identification label displayed on the shipper. Material is free from dust, dirt, and other foreign packaging material.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL					
Material	**Seal F/O 33mm/50ml Hook Type Cream Colour**				
Item Code	**1200009062**			**Department**	**Quality Control**
Specification No.	RLS2/QC/PM/SPC079	**Revision**	00	**Effective Date**	Jun 08,'07
Supersedes	None	**Dated**	NA	**Page No.**	Page 2 of 2
Next Review on	Maximum of 2 years from effective date				

Parameter for re-testing:

S. No.	Test	Specifications
01	Description	33 mm Flip top hook type aluminium Seal with Cream Colour plain Polypropylene disc.
02	Base Diameter	33.00 mm ± 0.30 mm
03	Formation	Seal is hook type. Hook designed such that it is hidden inside the polypropylene disc. Flip the disc to reach the circular hook. Hold the outer circular part of the hook and pull it up for hanging the bottle.
04	Packing	Seal packed in Double Polybag, in turn in shipper. Material identification label displayed on the shipper. Material is free from dust, dirt, and other foreign packaging material.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL

Material	**CARTON ALBUREL 20% IP 50ML-DOM**				
Item Code	1200007179			**Department**	Packaging Development
Specification No.	RLS/PM/CRT/001	**Revision**	00	**Effective Date**	04 Dec 07
Supersedes	None	**Dated**	NA	**Page No.**	1 of 2
Next Review on	Maximum of 2 years from effective date				

Storage conditions	Store at room temperature.
Re-testing period	2 Year

S. No.	Test	Specification
01	Description	ITC Safire graphic board lock bottom carton with matt BOPP lamination from outer side & OP medium coating on inner side. Free from visual defects & dust particles.
02	Length	52 ± 1 mm
03	Width	52 ± 1 mm
04	Height	86 ± 1 mm
05	Board Grammage with lamination	315.0 ± 15 g/m^2
06	Text matter	Must comply with approved artwork.
07	Colour Shades	Must comply with approved shade card.
08	Formation (Folding and Locking)	Should be proper.

Parameters for re-testing

S. No.	Test	Specification
01	Colour shade	Must comply with approved shade card.
02	Formation (Folding and Locking)	Should be proper.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL					
Material	**CARTON ALBUREL 20% IP 50ML-DOM**				
Item Code	1200007179			**Department**	Packaging Development
Specification No.	RLS/PM/CRT/001	**Revision**	00	**Effective Date**	04 Dec 07
Supersedes	None	**Dated**	NA	**Page No.**	2 of 2
Next Review on	Maximum of 2 years from effective date				

Packing

Bundles of 100 cartons should be packed in a plastic bag & placed in the corrugated shipper. Label should be pasted on individual shipper having following details, material name, SAP code, quantity per box & supplier name. Front panel of the carton should be pasted on every shipper for material identification purpose.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL

Material	Rubber Stopper Chloro Butyl 31mm Grey RFS-W/4432/50 3203 IV				
Item Code	1200010156			**Department**	Packaging Development
Specification No.	RLS/PM/RS/002	**Revision**	00	**Effective Date**	31 Mar 08
Supersedes	None	**Dated**	NA	**Page No.**	1 of 3
Next Review on	Maximum of 2 years from effective date				

Storage conditions	Store at room temperature
Re-testing period	1 Year

S. No.	Test	Specification
01	Description	Grey chlorobutyl ready for sterilization rubber stopper smoothly & evenly finished having three equidistance circles at the top surrounded by a circular ring. They are homogenous and practically free from flash and adventitious materials such as fibres, foreign particles and adhering rubber pieces. The design and dimension should be as per drawing no. WPK 3035 attached as an annexure.
02	Total Height	12.00 ± 0.38 mm
03	Rim Diameter	31.00 ± 0.3 mm
04	Rim Height	4.60 ± 0.3 mm
05	Plug Diameter (OD)	23.90 ± 0.2 mm
06	Sterilization test	The prepared stoppers are not soften, tacky and no visual change.
07	Shore hardness	50° ± 5 Shore A
08	Fragmentation test	Must comply as per IP (Not more than 15 fragments per 12 plugs)
09	pH of Aqueous extract	Must comply as per IP (Not more than 0.3 ml of 0.01 M sodium hydroxide or 0.6 ml of 0.01 M hydrochloric acid is required to change solution to blue or yellow respectively)
10	Light absorption	Must comply as per IP (Not more than 2.0 in the range of 220 -360 nm)

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL					
Material	**Rubber Stopper Chloro Butyl 31mm Grey RFS-W/4432/50 3203 IV**				
Item Code	1200010156			**Department**	Packaging Development
Specification No.	RLS/PM/RS/002	**Revision**	00	**Effective Date**	31 Mar 08
Supersedes	None	**Dated**	NA	**Page No.**	2 of 3
Next Review on	Maximum of 2 years from effective date				

S. No.	Test	Specification
11	Reducing substances	Must comply as per IP (The difference between the titration volumes is not more than 7.0 ml)
12	Heavy metals	Must comply as per IP (Complies with the limit test for heavy metals)
13	Residue on evaporation	Must comply as per IP (Not more than 4.0 mg)
14	Biological Test* a) Intravenous Injection Test b) Subcutaneous Injection Test	Must comply as per IP Must comply as per IP * Test to be carried out at FDA approved biological laboratories.

Parameters for re-testing

S. No.	Test	Specification
01	Fragmentation test	Must comply as per IP (Not more than 15 fragments per 12 plugs)
02	Shore Hardness	50° ± 5 Shore A
03	pH of Aqueous extract	Must comply as per IP (Not more than 0.3 ml of 0.01 M sodium hydroxide or 0.8 ml of 0.01 M hydrochloric acid is required to change solution to blue or yellow respectively)
04	Biological Test - Intravenous Injection	Must comply as per IP

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL					
Material	**Rubber Stopper Chloro Butyl 31mm Grey RFS-W/4432/50 3203 IV**				
Item Code	1200010156			**Department**	Packaging Development
Specification No.	RLS/PM/RS/002	**Revision**	00	**Effective Date**	31 Mar 08
Supersedes	None	**Dated**	NA	**Page No.**	3 of 3
Next Review on	Maximum of 2 years from effective date				

Packing

1000 stoppers should be packed in a sterilizable bag and such bag should be packed in shipper. Label should be pasted on individual shipper having following details, material name, SAP code, quantity per box and supplier name.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL					
Material	**BOTTLE GLASS USP-I MOLDED CLEAR 32mm 50ml-DIN**				
Item Code	1200007175			**Department**	Packaging Development
Specification No.	RLS/PM/GC/001	**Revision**	00	**Effective Date**	28 Mar 08
Supersedes	None	**Dated**	NA	**Page No.**	1 of 2
Next Review on	Maximum of 2 years from effective date				

Storage conditions	Store at room temperature.
Re-testing period	2 years

S. No.	Test	Specification
01	Description	Molded clear glass vial with 50 ml filling mark, USP Type-I, Free from dust, chipped neck, embedded/ glass particles.
02	Total Height	68.0 ± 0.9 mm
03	Body Diameter	46.0 ± 1.0 mm
04	Neck Outer Diameter	32.0 ± 0.3 mm
05	Neck Inner Diameter	22.7 ± 0.3 mm
06	Overflow capacity	68 ± 5 ml
07	Lip Thickness	7.0 ± 0.2 mm
08	Weight	55 ± 3 g
09	Powdered Glass Test	Not more than 1.0 ml of 0.020 N Sulfuric Acid
10	Arsenic test	Not more than 0.1 µg per g

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL					
Material	**BOTTLE GLASS USP-I MOLDED CLEAR 32mm 50ml-DIN**				
Item Code	1200007175			**Department**	Packaging Development
Specification No.	RLS/PM/GC/001	**Revision**	00	**Effective Date**	28 Mar 08
Supersedes	None	**Dated**	NA	**Page No.**	2 of 2
Next Review on	Maximum of 2 years from effective date				

Parameters for re-testing

S. No.	Test	Specification
01	Powdered Glass Test	Not more than 1.0 ml of 0.020 N Sulfuric Acid
02	Arsenic test	Not more than 0.1 µg per g

Packing

196 bottles should be packed in 5 ply corrugated shipper. Shippers should be properly wrapped with shrink film. Label should be pasted on individual shipper having following details, material name, SAP code, quantity per box and supplier name.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL

Material	**LABEL STICKER ALBUREL 20% IP 50ml – DOM**				
Item Code	1200008848			**Department**	Packaging Development
Specification No.	RLS/PM/LBL/002	**Revision**	00	**Effective Date**	04 Dec 07
Supersedes	None	**Dated**	NA	**Page No.**	1 of 2
Next Review on	Maximum of 2 years from effective date				

Storage conditions	Store at room temperature.
Re-testing period	2 Year

S. No.	Test	Specification
01	Description	Fasson paper roll form printed sticker label with UV varnish except overprinting area. Labels should be free from visual defects & foreign particles.
02	Length	120 ± 1 mm
03	Width	28 ± 1 mm
04	Paper GSM	80 ± 4 GSM
05	Adhesive GSM	20.0 ± 3.0 GSM
06	Release Paper GSM	Not less than 60 GSM
07	Core inner diameter	76.0 ± 1.0 mm
08	Outer diameter of roll	Should not be more than 175 mm
09	Text matter	Must comply with approved artwork.
10	Colour Shade	Must comply with shade card.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

SPECIFICATION - PACKAGING MATERIAL					
Material	LABEL STICKER ALBUREL 20% IP 50ml – DOM				
Item Code	1200008848			Department	Packaging Development
Specification No.	RLS/PM/LBL/002	Revision	00	Effective Date	04 Dec 07
Supersedes	None	Dated	NA	Page No.	2 of 2
Next Review on	Maximum of 2 years from effective date				

Parameters for re-testing

S. No.	Test	Specification
01	Colour Shade	Must comply with approved shade card.
02	Adhesive GSM	20.0 ± 3.0 GSM

Packing

An individual roll of diameter not more than 175 mm with 76 mm core should be wrapped in plastic film / bag & such wrapped rolls should be placed in the corrugated shipper. Corrugated shipper should be pasted with a sample label for identification.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

CERTIFICATE OF ANALYSIS

CERTIFICATE OF ANALYSIS

Format No: RLS/QC/COA/AR/020/00 **Analytical Reference No.: AR/FP/08/6169**

Name of the product : AlbuRel™ (Human Normal Albumin IP, 20 % Solution) – 50 mL

Lot Number : 2B20000039

Mfg. Date : MAY. 2008 **Exp. Date : APR. 2011**

Sr. No.	TEST	SPECIFICATION	RESULT
1.	Description	Clear, slightly viscous liquid, ranging in colour from almost colourless to greenish-yellow or amber colour.	Clear, slightly viscous, greenish yellow colour liquid.
2.	Identification A) Ouchterlony Test B) Electrophoresis	 Shall Comply Shall Comply	 Complies Complies
3.	Acidity & Alkalinity	Between 6.7 and 7.3	6.99
4.	Alkaline Phosphatase	Not more than 0.1 U / g protein	0.053 U / g
5.	Haem Content	Absorbance at 403 nm shall be not more than 0.15	0.067
6.	Denatured Protein	Not more than 5.0 %	0.492 %
7.	Protein Composition	Not less than 95 % of the total protein as Albumin	Complies
8.	Stability Of Solution	Must comply with the test for stability	Complies
9.	Sterility Test	Must comply with the test for sterility	Complies
10.	Pyrogens	Must comply with the test for pyrogens	Complies
11.	Abnormal Toxicity	Must comply with the test for abnormal toxicity	Complies
12.	Assay (Protein)	Between 19 % and 21 %	20.66 %
13.	Assay (Sodium)	Between 95 % To 105 % of Label Claim but not exceeding 160 mmol/L	129.2 mmols/L
14.	Assay (Potassium)	Between 95 % To 105 % of Label Claim but not exceeding 2 mmol/L	0.2 mmols/L
15.	Aluminium Content	Not more than 200 µg/L	27 µg/L
16.	HIV I & II by PCR*	Must not be detected	Not detected
17.	HBV by PCR*	Must not be detected	Not detected
18.	HCV by PCR*	Must not be detected	Not detected

**Inhouse Tests*

***Conclusion** : The following Lot No. 2B20000039 Complies/~~Does Not Comply~~ as per IP1996.*

Analysed By : Checked By : Approved By :

Date : Date : Date :

Issued on :

GENERAL TESTING PROCEDURE					
Description	Parvo Virus DNA PCR			Department	Quality Control
GTP No.	RLS2/QC/GTP014	Revision	01	Effective Date	25 Aug 08
Supersedes	00	Dated	22 Nov 07	Page No.	9 of 9
Distribution	None				
Next Review on	Max. 2 years from Effective date				

Revision History	
Changes	1. Under Safety Precautions the term "sample processing area" is mentioned instead of "sample processing room". 2. All types of typographical errors corrected. 3. An additional Note about verification of the Positive Control used is added. 4. The following changes are made in the annexure QC/GTP014/A01 4.1 Done by, Reviewed By with Date and Page Number has been added in all the pages. 4.2 Equipment codes required for all the equipments have been included in the annexure.
Reason for Changes	1. Sample processing room is not mentioned as it is not available all the time instead sample processing area is mentioned. 2. Positive Controls obtained from MME laboratory needs to be verified before being used as Controls. 3. Changes made to update the GTP and Annexure for the improvement of document.

	Prepared by	Verified by	Approved by
Signature			
Name			
Designation			
Date			

14 January, 2008

To Quality Control Group

From Dr. Vikram Paradkar,
Vice President, Process Development

Reference Stability assessment for Alburel™ USP product

Introduction

Alburel™, the 20% albumin solution for parenteral use, has been manufactured by Reliance Biopharmaceuticals for last 3 years for commercial distribution in India. This albumin solution is produced from the Fraction V paste generated during plasma fractionation. Depending on the pharmacoepia requirements, the final composition of the albumin solution consists of a combination of three key ingredients; sodium caprylate, N-acetyl-DL-tryptophan and sodium chloride. Reliance Biopharmaceuticals intends to produce Alburel™ meeting USP specifications (listed in FDA Code of Federal Register, section 640). The target USP requirements for final composition are 200 gm/L albumin, 16 mM sodium caprylate, 16 mM N-acetyl-DL-tryptophan and 100 mM of sodium chloride. This document provides a summary of stability studies performed to date on various types of Alburel™ produced at Reliance Biopharmaceuticals in support of shelf-life claim for Alburel™ USP.

Stability indicating parameters

Following Table shows the stability indicating parameters employed for the stability studies and the corresponding acceptance criteria.

Parameter	Acceptance Criteria	Rationale
Appearance	Clear, viscous, slightly yellowish solution	To detect phenomena such as protein precipitation/denaturation via formation of turbidity and color formation due to oxidation
pH	6.7 – 7.3	Solution pH can drift due to protein oxidation
Total protein	19 – 21%	To detect any loss of protein due to precipitation or adsorption
Haem content	Absorbance at 403 nm NMT 0.15	Haem is an impurity present in albumin that originates from haemoglobin.
Protein purity	Complies with reference standard	To detect generation of aggregates and clipped species
Denatured protein content	NMT 5%	Albumin can get denatured during storage and form aggregates that can be detected and quantified.

Stability Data

Part I: Pilot scale stability study

Three consecutive Alburel™ batches were produced in May 2006 with a target composition of 200 gm/L albumin, 16 mM sodium caprylate, 16 mM N-acetyl-DL-tryptophan and 50 mM sodium chloride. Data up to 18 months at 25°C and 12 months at 40°C are analyzed to date. The 40°C study was concluded at 12 months duration. The data is summarized in Appendix I.

Part II: Commercial scale stability study

Three consecutive Alburel™ batches were produced in Aug 2007 with a target composition of 200 gm/L albumin, 32 mM sodium caprylate and 50 mM sodium chloride. Data up to 3 months at 2-8°C, 25°C and 40°C are analyzed to date. The data is summarized in following tables.

Analysis

Alburel™ produced at pilot and commercial scale and consisting of slightly different overall compositions demonstrates excellent stability profile at the recommended storage temperature as well as the higher temperature of 40°C employed to assess stability under potentially accelerated degradation conditions. No adverse trends were observed for any of the quality attributes of the protein (overall concentration, composition and denatured protein content). The pH of the solution showed a slight upward trend (as a function of time but it was well within the specification of 6.7 to 7.3 and predicted to remain in that range at the end of the shelf life.

Conclusion

The stability assessments indicate that Alburel™ produced to meet USP specifications for overall composition will remain stable at the recommended storage temperature of 25°C for the entire shelf life duration of 36 months.

Appendix I: Pilot Scale Stability Data

Batch: ALT/RD/001

Test Period	Appearance	pH	Total Protein (%)	Haem Content	Protein purity
Storage Temperature: 25°C					
Initial	Complies	6.9	20.3	0.033	Complies
1 Month	Complies	6.9	20.4	0.030	Complies
2 Months	Complies	7.0	20.1	0.027	Complies
3 Months	Complies	7.0	20.4	0.029	Complies
6 Months	Complies	6.9	20.2	0.031	Complies
9 Months	Complies	7.0	20.0	0.033	Complies
12 Months	Complies	7.0	20.5	0.035	Complies
18 Months	Complies	7.1	20.2	0.033	Complies
Storage Temperature: 40°C					
Initial	Complies	6.9	20.3	0.044	Complies
I month	Complies	6.8	20.3	0.047	Complies
2 months	Complies	6.9	20.1	0.059	Complies
3 Months	Complies	6.9	20.1	0.043	Complies
6 Months	Complies	6.9	20.2	0.051	Complies
9 Months	Complies	7.0	20.4	0.048	Complies
12 Months	Complies	7.1	19.9	0.035	Complies

Batch: ALT/RD/002

Test Period	Appearance	pH	Total Protein (%)	Haem Content	Protein purity
Storage Temperature: 25°C					
Initial	Complies	6.8	20.1	0.025	Complies
1 Month	Complies	6.9	20.1	0.031	Complies
2 Months	Complies	7	20.4	0.029	Complies
3 Months	Complies	6.8	20.3	0.032	Complies
6 Months	Complies	6.8	20.1	0.033	Complies
9 Months	Complies	6.9	20	0.032	Complies
12 Months	Complies	6.8	20.5	0.038	Complies
18 Months	Complies	6.9	20.8	0.039	Complies
Storage Temperature: 40°C					
Initial	Complies	6.8	20.1	0.043	Complies
I month	Complies	6.9	20.3	0.048	Complies
2 months	Complies	7	20.4	0.06	Complies
3 Months	Complies	6.9	20.4	0.044	Complies
6 Months	Complies	6.9	20.1	0.057	Complies
9 Months	Complies	7	20.6	0.051	Complies
12 Months	Complies	7	20.5	0.063	Complies

Batch: ALT/RD/003

Test Period	Appearance	pH	Total Protein (%)	Haem Content	Protein purity
Storage Temperature: 25°C					
Initial	Complies	6.8	20.2	0.023	Complies
1 Month	Complies	6.9	20.1	0.031	Complies
2 Months	Complies	7.0	20.2	0.050	Complies
3 Months	Complies	6.8	20.2	0.035	Complies
6 Months	Complies	6.9	20.3	0.037	Complies
9 Months	Complies	7.0	20.0	0.025	Complies
12 Months	Complies	7.0	20.8	0.036	Complies
18 Months	Complies	6.9	19.9	0.042	Complies
Storage Temperature: 40°C					
Initial	Complies	6.8	20.1	0.052	Complies
1 month	Complies	6.8	20.3	0.051	Complies
2 months	Complies	6.8	20.3	0.057	Complies
3 Months	Complies	6.8	20.2	0.048	Complies
6 Months	Complies	7.0	20.1	0.054	Complies
9 Months	Complies	6.8	19.8	0.052	Complies
12 Months	Complies	7.0	20.5	0.049	Complies

Appendix II: Commercial scale stability data

Batch: 4B40000267

Study Duration	Physical appearance	pH	Denatured protein (NMT 5%)	Protein composition	Total Protein
2-8°C					
Initial	Complies	6.83	0.728%	Complies	20.20%
0.5 month	Complies	6.94	0.536%	Complies	20.74%
1 month	Complies	6.93	0.530%	Complies	20.50%
2 month	Complies	6.98	0.536%	Complies	20.55%
3 month	Complies	6.98	0.544%	Complies	20.98%
25°C					
Initial	Complies	6.83	0.728%	Complies	20.20%
0.5 month	Complies	6.97	0.672%	Complies	20.76%
1 month	Complies	6.99	0.662%	Complies	20.87%
2 month	Complies	6.99	0.574%	Complies	20.64%
3 month	Complies	7.02	0.580%	Complies	20.72%
40°C					
Initial	Complies	6.83	0.728%	Complies	20.20%
0.5 month	Complies	6.93	0.604%	Complies	20.59%
1 month	Complies	6.94	0.588%	Complies	20.50%
2 month	Complies	6.98	0.536%	Complies	20.55%
3 month	Complies	6.99	0.612%	Complies	20.89%

Batch: 4B40000268

Study Duration	Physical appearance	pH	Denatured protein (NMT 5%)	Protein composition	Total Protein
2-8°C					
Initial	Complies	6.92	0.582%	Complies	20.47%
0.5 month	Complies	6.99	0.546%	Complies	20.57%
1 month	Complies	7.02	0.530%	Complies	20.59%
2 month	Complies	7.04	0.574%	Complies	20.46%
3 month	Complies	7.02	0.612%	Complies	20.98%
25°C					
Initial	Complies	6.92	0.582%	Complies	20.47%
0.5 month	Complies	7	0.536%	Complies	20.84%
1 month	Complies	7.01	0.662%	Complies	20.78%
2 month	Complies	7.02	0.574%	Complies	20.55%
3 month	Complies	7.04	0.544%	Complies	20.72%

40°C					
Initial	Complies	6.92	0.582%	Complies	20.47%
0.5 month	Complies	6.91	0.672%	Complies	20.68%
1 month	Complies	6.93	0.588%	Complies	20.71%
2 month	Complies	6.94	0.646%	Complies	20.64%
3 month	Complies	6.96	0.726%	Complies	20.89%

Batch: 4B40000269

Study Duration	Physical appearance	pH	Denatured protein (NMT 5%)	Protein composition	Total Protein
2-8°C					
Initial	Complies	6.88	0.728%	Complies	20.02%
0.5 month	Complies	6.91	0.614%	Complies	20.59%
1 month	Complies	6.94	0.680%	Complies	20.89%
2 month	Complies	6.99	0.574%	Complies	20.55%
3 month	Complies	7.02	0.680%	Complies	20.98%
25°C					
Initial	Complies	6.88	0.728%	Complies	20.02%
0.5 month	Complies	6.95	0.682%	Complies	20.66%
1 month	Complies	6.96	0.680%	Complies	20.99%
2 month	Complies	7.02	0.646%	Complies	20.64%
3 month	Complies	7.04	0.680%	Complies	20.81%
40°C					
Initial	Complies	6.88	0.728%	Complies	20.02%
0.5 month	Complies	6.92	0.614%	Complies	20.83%
1 month	Complies	6.91	0.606%	Complies	20.89%
2 month	Complies	6.98	0.574%	Complies	20.82%
3 month	Complies	6.99	0.652%	Complies	20.89%

MATERIAL SAFETY DATA

SHEET

FOR

INDEX

SECTION I : PRODUCT AND COMPANY IDENTIFICATION

Product Code	:	SAP Code No. 4B40000001 and so on.
Product Name	:	AlbuRel TM
Manufacturer Name and Address	:	Reliance Life Sciences Pvt Ltd. Plant I, Sadhana Mills Compound, Pandurang Budhkar Marg, Worli, Mumbai – 400 018. Maharashtra, India.
Telephone Numbers	:	+91 (022) 66120808
Responsible Person and Contact Number	:	Umesh B G Head QA +91 (022) 67678600 +91 9967656219

SECTION II : COMPOSITION / INFORMATION ON INGREDIENTS

Sr. No.	Common Name	CAS No.	Comments
1	Human Albumin (5%, 20%, 25%)	NA	Active ingredient
2	Sodium Caprylate (< 0.5%)	NA	Stabilizer
3	Sodium Chloride, USP (<1%)	7467 – 14 – 5	Osmotic balance adjustment
4	Sodium Bicarbonate, USP	144 – 55 – 8	pH adjustment
5	Sodium Hydroxide, ACS	1310 – 73 – 2	pH adjustment
6	Glaical Acetic Acid, ACS	64 – 19 - 7	pH adjustment

7	Water For Injection, USP	7732 – 18 – 5	Volume adjustment

SECTION III : HAZARDOUS IDENTIFICATION

EMERGENCY OVERVIEW		
Route(s) of Entry:		
Skin contact	:	No data. No adverse health effects reported nor anticipated
Skin Absorption	:	This product is not absorbed through skin
Eyes	:	No data. No adverse health effects reported nor anticipated
Ingestion	:	Not intended for oral use. Expected to be relatively non-toxic if ingested.
Potential Health effects:		
This product has been prepared form the pooled plasma of healthy adult donors. Each plasma donations has been tested for the absence of antibodies against HIV-1, HIV-2 and Hepatitis C, as well as for the absence of HIV antigen and Hepatitis B Surface antigens. In addition, the product underwent a minimum of two different virus reduction procedures. However, the risk of infectivity due to known or as yet unknown pathogens cannot be totally eliminated from this product. No adverse health effects anticipated with normal handling and use in appropriate medical setting. Medical implications of therapeutic use are described in product package insert or may be found in the Physician's Desk reference.		
Chronic Effects / Carcinogenicity:		
None known or anticipated under normal handling and exposure conditions.		

SECTION IV : FIRST AID MEASURES

EMERGENCY AND FIRST AID PROCEDURES:

Eyes	:	Flush with water for 15 – 20 Minutes. If irritation develops, seek medical attention.
Skin	:	Wash with soap and water. If irritation or other symptoms develop, seek medical attention.
Ingested	:	Rinse from mouth and seek medical guidance. Induce vomiting only as directed by medical Personnel. Never give anything by mouth to an unconscious person.
Inhalation	:	If inhaled, remove to fresh air. Seek medical attention if symptoms develop or if breathing is difficult.

SECTION V : FIRE FIGHTING MEASURES

FLAMMABLE LIMITS		
Flash Pt	:	NA
Flammable limits in Air- Lower	:	NA
Flammable limits in Air- Upper	:	NA
Auto-Ignition Temperature	:	NA
General Hazards	:	Product is not flammable. The only potential hazard would involve packaging material.
Fire Fighting	:	Packaging material fires may be extinguished with

Extinguishing Media		water, carbon dioxide, or dry chemical.
Fire Fighting Instructions	:	Fire Fighting Personnel should respond with appropriate protective clothing, firefighting gear, and breathing equipment as trained. All other personnel should exit the area and proceed to the gathering point in an area unaffected by the fire and smoke.
Hazardous Combustion Products	:	Packaging material fire may produce Carbon monoxide and other gaseous asphyxiants plus airborne particulate matter.

SECTION VI : ACCIDENTAL RELEASE MEASURES

Large Spill	:	Absorb spills with material suitable for aqueous solutions and dispose in solid waste container, or mop spilled material with detergent/water solution and dispose in sanitary sewer. Ventilate area, If desired.
Small Spill	:	Clean area of spill with wetted toweling and dispose in solid waste container, or follow procedure for large spills.

SECTION VII : HANDLING AND STORAGE

Special Handling
Prevent physical damage to package to avoid breakage and spilling.

Special Storage
Store in accordance with the conditions specified in the product package insert.

SECTION VIII : EXPOSURE CONTROLS/PERSONAL PROTECTION

Eye Protection	:	None required to provide protection against the product. Eye protection may be required by procedure of administration.
Skin Protection	:	None required for protection against the product. Medical-grade examination or surgical gloves may be required by procedure of administration.
Respiratory Protection	:	None required
Engineering Controls	:	Not applicable.

SECTION IX : PHYSICAL AND CHEMICAL PROPERTIES

Physical form of pure concentrate	:	Clear aqueous solution
Physical form	:	Clear aqueous solution
Color	:	Ranges from almost colorless to greenish-yellow to amber color.
Odor	:	Unspecified
Boiling Point	:	Unspecified
Melting Point	:	Unspecified
Freezing Point	:	Unspecified
pH	:	6.9 ± 0.5

Solubility in Water	:	Complete
Specific Gravity	:	Unspecified
Decomposition Temperature	:	Unspecified
Odor Threshold	:	Unspecified
Evaporation Rate	:	Unspecified
Vapor Rate	:	Unspecified
Vapor Pressure	:	Unspecified
Vapor Density	:	Unspecified

SECTION X : STABILITY AND REACTIVITY

Stability	:	Stable for period indicated on the label when stored at conditions specified in product package insert.
Incompatibility	:	No known incompatibilities
Hazardous Decomposition Products	:	No known Hazardous Decomposition Products
Hazardous Polymerization	:	Hazardous Polymerization will not occur
General Information	:	No additional information

SECTION XI : TOXICOLOGICAL INFORMATION

This is a pasteurized and sterile, filtered aqueous solution containing human albumin, stabilizers, and osmotic and buffering agents. It is not exceeded to be toxic by ingestion nor a skin/eye irritant. More comprehensive and detailed product information is contained in the product package insert or may be found in the Physicians' Desk Reference.

SECTION XII : ECOLOGICAL INFORMATION

No ecological damage or persistence in the environment expected under normal conditions of use or with proper disposal. Environmental fate and transport of this product have not been studied.

SECTION XIII : DISPOSAL CONSIDERATIONS

Disposal Information
Not classified as hazardous waste. Observe all federal, state and local regulations.
Waste Disposal Methods
Waste must be disposed in accordance with federal, state and local environmental regulations. Uncontaminated product may be disposed by flushing down the sanitary sewer, or by mixing with a liquid sorbent and then placing mixture ini the solid waste container for disposal. Incineration is the preferred method of disposal for any contaminated product.

SECTION XIV : TRANSPORT INFORMATION

Proper Shipping Name	:	Not Regulated
Hazard Class	:	Not Regulated
Transport of Hazardous Material Description	:	Domestic DOT label: None; International (IMO) label: Drug/Medicines. Ship according to DOT and/or IATA regulations.

MATERIAL SAFETY DATA SHEET FOR Alburel ™ (HUMAN ALBUMIN I.P. 20 %)	

SECTION XV : REGULATORY INFORMATION
Alburel TM (Human Albumin I.P. 20%)

TSCA:
This material is a biological product regulated by the United States Food and Drug Administration (FDA)
CERCLA:
NA
SARA 302
NA
SARA 313
NA

SECTION XVI : OTHER INFORMATION
Alburel TM (Human Albumin I.P. 20%)

Prepared By	:	Reliance Life Sciences Pvt. Ltd.
Approved By	:	Indian Food and Drug Administration (FDA)
Approved Date	:	May 05,'2005
Supersedes Date	:	NA
Declaration:		
The information contained herein is based upon data considered true and accurate. Reliance Life Sciences Pvt. Ltd. Makes no warranties, express or implied, as to the adequacy of the information contained herein. This information is offered solely for the user's consideration, investigation, and verification. Report to the manufacture any allegations of health effects resulting from handling or accidental contact with this material.		

ARTWORKS:
PACKAGE INSERT
LABEL
CARTON

For the use of a Registered Medical Practitioner or a Hospital only

COMPOSITION
Each bottle contains:

Concentrations available (%)	20%	5%
Pack size	50ml/100ml	50ml/100ml
Total Protein	200 g/l	50 g/l
Sodium Caprylate	6.65 g/l	1.66 g/l
Na^+ not more than	160 mM	160 mM
K^+ not more than	2 mM	2 mM
Aluminum	≤ 200 µg/l	≤ 200 µg/l

AlbuRel™ does not contain any antimicrobial agents

CLINICAL PHARMACOLOGY
Albumin is a highly soluble, globular protein (molecular weight 66,500), accounting for 70-80% of the colloid osmotic pressure of plasma. Therefore, albumin is important in regulating the osmotic pressure of plasma. Albumin 20% solution will increase the circulating plasma volume by four times the volume infused. This extra fluid reduces haemoconcentration and decreases blood viscosity. The degree and duration of volume expansion depend upon the initial blood volume. When treating patients with diminished blood volume, the effect of infused albumin may persist for many hours. The haemodilution lasts for a shorter time when albumin is administered to individuals with normal blood volume. Albumin also functions as a transport protein and binds to naturally occurring, therapeutic and toxic materials in the circulation. The binding properties of albumin may, in special circumstances, provide an indication for its clinical use.

Albumin is distributed throughout the extracellular water and more than 60% of the body albumin pool is located in the extravascular fluid compartment. The total body albumin in a 70 Kg man is approximately 320 g; it has a circulating life span of 15-20 days, with a turnover of approximately 15 g per day.

CLINICAL INDICATIONS
Albumin (Human Albumin), may be given intravenously without dilution or it may be diluted with normal saline or 5% glucose before administration. The addition of one volume of albumin solution to four volumes of normal saline or 5% glucose gives a solution approximately isotonic and iso-osmotic with citrated plasma. When undiluted albumin solution is administered in patients with normal blood volume, the rate of infusion should be slow enough (1 ml per minute) to prevent too rapid expansion of plasma volume.

I. Hypo-volaemic shock: Albumin is indicated in the treatment of hypo-volaemic shock associated with blood loss, trauma and surgical procedures. Albumin solutions are an accepted form of resuscitation, although crystalloids are the initial fluid of choice.

II. Burns: Albumin is used for severe burns (>15% body surface area) after the first 24 hours, if hypo-proteinaemia develops and/or to maintain plasma volume.

III. Hypo-proteinemia: Albumin is indicated in the treatment of hypo-proteinaemia caused by a loss of plasma proteins. Loss of plasma proteins may occur through decreased absorption in gastrointestinal disorders, inadequate synthesis in chronic liver diseases or excessive urinary catabolism in chronic liver diseases. This loss of proteins leads to oedema, secondary to a fluid shift from the intravascular space to the interstitium and a compensatory increase in salt and water retention. Albumin serves to restore colloid osmotic pressure and in, conjunction with a diuretic, promotes diuresis.

IV. Ascites: Albumin may be used to maintain cardiovascular function following removal of large volumes of ascitic fluid in patients suffering from ascites.

V. Plasma exchange/dialysis: Albumin 20% may be used as an adjunct in patients who are undergoing long-term haemo-dialysis and are susceptible to shock and hypo-tension, or in dialysis patients who are hypo-volaemic and may not tolerate large volumes of crystalloid infusion as treatment for shock or hypo-tension.

PRODUCT SAFETY
The manufacturing process for *AlbuRel*™ uses plasma collected from approved blood banks where the donors are screened for their history as per guidelines laid down by the regulatory authorities. Their blood is screened for the mandatory infectious diseases. These are repeat donors whose samples are quarantined and retested. Only on being declared negative to HbsAg, HIV I & II antibodies and HCV RNA the plasma is used for processing.

The manufacturing procedure incorporates Heat Pasteurization (60° C for 10 hours), which inactivates viruses. After manufacturing, the product is tested by suitable methods to show freedom from viruses like HIV, HBV, HCV, Parvovirus and HAV. Multiple steps have been employed to assure product safety hence there is a very remote probability that unknown infectious agents may be present in these products like newer emerging viruses and theoretical CJD (Creutzfeldt Jakob disease). The process parameters, characterisations and final product quality are designed such, that they meet the regulatory requirements. Records of blood donors whose plasma have been used for manufacturing of this product has been maintained for at least ten years at the site of origin.

DOSAGE AND DIRECTIONS FOR USE
Albumin solutions need not be given through a filter. No compatibility testing (cross-matching) need to be performed since no ABO blood group antibodies are present.

Albumin is hyperosmotic and should be given by slow intravenous infusion at a rate of about 1 ml per minute. The rate of infusion and the total volume of albumin administered ultimately must be guided by the haemodynamic response of the patient and the clinical indication for which it has been prescribed.

Transfusions of whole blood or packed red blood cells may be necessary following administration of large volumes of albumin to restore the haemoglobin concentration and prevent anaemia.

Dosage guidelines:
The following dosages are included as a guideline only. The clinician should determine appropriate therapy after clinical assessment of the patient. Serum albumin values alone should not be used to determine dosage.

Hypovolaemia:
Adult dosage:
Start with 20 g of infusion and look for clinical response. May be repeated if necessary.

Paediatric dosage:
Start with 0.5 to 1 g per Kg body weight and repeat if clinical response is not adequate.

Burns:
Adult and Paediatric dosage:
Start with 20 g of Albumin, ensure adequate Plasma volume and repeat a similar dose after 24 hours.

Hypoproteinaemia:
Two / three infusions of 20 g may be given with monitoring of protein levels.

Ascites:
Preferable to use 20 g to start with, but requires protein and fluid monitoring.

Plasma exchange and Dialysis:
25 g as an intravenous infusion not exceeding 30 ml / min during plasma exchange.

OVERDOSAGE:
Rapid infusion and large volumes may cause hypervolaemia. The physician is recommended to look for clinical signs and symptoms and stop infusion immediately.

SIDE EFFECTS
Allergic or pyrogenic reactions are characterized primarily by fever and chills. Rash, nausea, vomiting, tachycardia, hypo-tension and increased salivation have also been reported. Should an adverse reaction occur, stop the infusion immediately for a period of time, which may result in the disappearance of the symptoms. If administration has been stopped and the patient requires additional Albumin, material from a different lot should be used.

Albumin, particularly if administered rapidly, may result in vascular overload with resultant pulmonary edema.

WARNINGS AND PRECAUTIONS
General:
Solutions of ALBUMIN (*AlbuRel*™) should not be used if they appear turbid or if there is sediment in the bottle. Contents must not be used more than four hours after the container has been penetrated. Discard unused portion.

Medical products prepared from human plasma carry a very remote risk of transmission of infective agents that are reduced by several methodologies.

Albumin should be administered with caution to patients with low cardiac reserve.

Rapid infusion may cause vascular overload with resultant pulmonary edema. Patients should be closely monitored for signs of increased venous pressure.

Patients with marked dehydration require administration of additional fluids. Albumin may be administered with the usual dextrose and saline intravenous solutions. However, solutions containing protein hydrolysates or alcohol must never be infused through the same administration set in conjunction with *AlbuRel*™ since these combinations may cause precipitation of proteins.

Pregnancy Category C:
There are no known adverse reports on Albumin usage in pregnant women in any trimester or to the foetus.

Paediatric Use:
Appropriate dose based on body weight is not known to cause any undesirable effect.

CONTRAINDICATIONS
Albumin is contraindicated in patients with severe anaemia or cardiac failure in the presence of normal or increased intravascular volume.

The use of Albumin is contraindicated in patients with a history of allergic reactions to Albumin preparations.

PRESENTATION
AlbuRel™ is available as 20% intravenous infusion in two packs, containing 20 g and 10 g of Human Albumin per 100 ml and 50ml respectively.

AlbuRel™ is also available as 5% intravenous infusion in two packs, containing 5 g and 2.5 g of Human Albumin per 100 ml and 50ml respectively.

STORAGE
Store the container at or below 25°C. Do not freeze. Protect from light. Keep out of reach of children.

SHELF LIFE
3 years from the date of manufacturing.

Each bottle contains :

Total protein	200 g/l
Sodium caprylate	6.65 g/l
Na^+ Not more than	160 mM
K^+ Not more than	2 mM
Aluminium	≤ 200 µg/l

Store at or below 25°C.
Do not freeze.
Contents must not be used more than four hours after the container has been penetrated and any remnant portion must be discarded.
Suitable for administration to patients undergoing dialysis or to premature infants.
Refer to pack insert prior to use.
Do not use if solution is turbid.
Source : Human Plasma
Non-reactive to HBs Antigen, HCV, HIV I & II **antibodies and negative for HCV RNA by PCR.**

50 ml

Mfg. Lic. No. : 28E-3
Lot No. :
Mfg. Date :
Exp. Date :

200 % Enlarged Size

50 ml

10 g

Each bottle contains :

Total protein	200 g/l
Sodium caprylate	6.65 g/l
Na^+ Not more than	160 mM
K^+ Not more than	2 mM
Aluminium	≤ 200 µg/l

Source : Human Plasma

Non-reactive to HBs Antigen, HCV, HIV I & II antibodies and negative for HCV RNA by PCR.

Store at or below 25°C.

Do not freeze.

Contents must not be used more than four hours after the container has been penetrated and any remnant portion must be discarded.

Suitable for administration to patients undergoing dialysis or to premature infants.

Refer to pack insert prior to usage.

Do not use if solution is turbid.

50 ml

10 g

1200009314

Mfg. Lic. No. : 28E-3

Lot No. :

Mfg. Date :

Exp. Date :

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