Validation Guide

LeRybow® 1/2 Single-use liner bag

CATALOGUE

1. Introduction

Single-use systems are widely used in biopharmaceutical process operations, involving the commercial production of pharmaceutical products such as vaccines, recombinant proteins and monoclonal antibodies and the mixing or storage of buffer, culture media and terminal preparations. LeRybow®1/2 liner bag produced by LePure Biotech is an important product of the single-use system. The qualification, manufacture and release of the product meet the strict product verification protocol and the requirements of the pharmaceutical manufacturing management standard (cGMP). Besides, the product pass the quality control test, which can be used for the biopharmaceutical industry and provide safe, reliable single-use processes to end users.

This Validation Guide describes the production conditions, quality standards, quality control tests and physicochemical characteristic of LeRoybow®1/2 liner bag made of ULDPE.

1.1 Manufacturing Site

At present, LePure has 2 production bases.

The Minyi Road factory was put into operation in 2019 with the total area of 7911.11m^2 and the Class C clean area is 900 $m^{2;}$

The Lingang factory was put into operation in 2020 with the total area of 10078m^2, the Class D is 450m^2, the Class C is 3400m^2 and the Class C+A is 600m^2.

1.2 GMP Quality Assurance

Our quality system is ISO 9001 compliant and applies across all production facilities. The company regards ISO 9001 as its fundamental quality management system. LeRybow® 1/2 liner bag meet stringent performance and industry safety standards as described in this validation guide. Besides, the key manufacturing operations are conducted in an ISO 7 or ISO 8 compliant cleanroom environment. Ongoing staff training and qualifications to ensure employees have awareness of the required standards. Employee qualifications are measured through rigorous initial screening and ongoing competency testing. The quality and safety of products are constrained and guaranteed by supplier management policies, such as incoming material quality control, process monitoring and finished product inspection and release. Our change control management and centralized documentation systems ensure product and process consistency and achieve continuous improvement.

1.3 Gamma irradiation sterilization

According to the provisions of ISO 11137 Parts 1&2:2013, use the validation method of VDmax with the minimum sterilization dose (such as 25kGy or 40kGy) and the method of the sterilization cycle validation. After selecting representative product series to determine the average microbial load level to carrying out experiments, final confirmation is that the gamma irradiation dose range of 25-40kGy can guarantee the sterility assurance level SAL=10^{-6}

1.4 Summary of Validation of Single-Use Systems

- Mechanical properties
  ASTM D882: Tensile Strength
  ASTM D4169-05:Drop test
- Gas permeability
  ASTM D1434: Oxygen Transmission Rate
  ISO 15106-2: Water Vapor Transmission Rate
- Biocompatibility
  ISO 10993-4, ISO 10993-5, ISO 10993-6, ISO 10993-10, ISO 10993-11, USP<87>, USP<88> biocompatibility testing
- USP<788>: Particulate Matter
  ≥10μm : ≤ 10/mL
  ≥ 25μm : ≤ 1/mL
- Bacterial endotoxin tests
  Bacterial endotoxin < 0.125 EU/mL
- Sterilization test
  ISO 11137 Parts 1&2:2013: Gamma irradiation sterilization
- Shelf-life verification

2. Production and Quality

2.1 Personnel

LePure Biotech has a group of production, quality, management and technical personnel who have the corresponding professional knowledge of single-use systems in biological processes. Personnel at all levels of the company have undergone strict selection and training. All quality authorized personnel meet the relevant personnel qualification requirements of ISO 9001:2015 "Quality Management System Requirements", and have relevant professional knowledge and work experience. So they can perform their duties in production and quality management.

2.2 Plant and facilities

The air cleanliness level of the production environment of LePure Biotech single-use system is not lower than the air cleanliness level of the single-use system process used in the production of pharmaceuticals and complies with T/CNPPA 3005-2019 "Guidelines for the Production Quality Management of Pharmaceutical Packaging Materials" No.6 Requirements for plant and facilities in chapter.

2.3 Material Control

According to the characteristics of the single-use system, the selection criteria, management methods and audit specifications for suppliers have been established. Moreover, the quality of raw

materials used in LeRybow®1/2 liner bag is fully checked to avoid the use of toxic and harmful additives, so as to control risks from the source. All raw materials are free of TSE/BSE and conform to USP class VI. All raw materials and auxiliary materials are animal-free and traceable. So it can be traced.

2.4 File Management

2.4.1 Process specification

LeRybow®1/2 liner bag has corresponding production process regulations, including process flow, key equipment operation requirements, intermediate control methods and qualification standards. It also has material balance calculation methods and limits. The revision review and approval process of process regulations are stipulated in accordance with relevant procedures.

2.4.2 Batch production records

Each batch of LeRybow®1/2 liner bag has a corresponding batch production record to record the key process parameters of production. Besides, the production history of this batch of products and the situation related to product quality can be traced.

2.5 Production process control

2.5.1 Batch division and batch number compilation

The production of LeRybow®1/2 liner bag has established operating procedures for product batches to ensure the uniformity and consistency of product quality and characteristics of the same batch. Besides it has formulated standard operating procedures for batch numbers and production dates. Each batch of products has create a unique lot number.

2.5.2 Identification and traceability

The materials used in the production of LeRybow®1/2 liner bag, the intermediate products or the containers of the products to be packaged, the main equipment and the necessary production workshops are all labeled and marked or the names, specifications and batch numbers of the materials in production are marked in other ways. Trace the whole process of product realization.

2.5.3 Clean production management

Clean production management complies with the requirements of 10.3 in T/CNPPA 3005-2019 "Guidelines for Quality Management of Pharmaceutical Packaging Materials".

2.5.4 Product sterilization

The radiation sterilization process of LeRybow® 1/2 process bags has been verified and can reach a sterility assurance level (SAL) of 10^{-6}, and the management of the radiation sterilization process complies with ISO 11137 Parts 1&2:2013.

3. Thin film properties

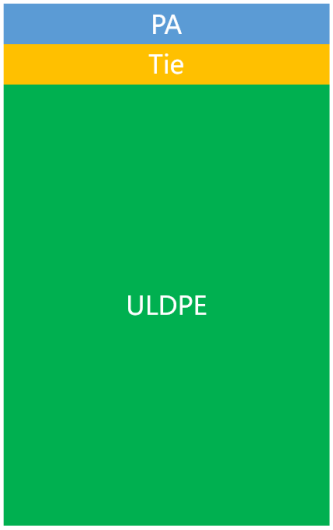
3.1 Thin film structure

Multi-layer co-extrusion film, using multi-layer structure of different materials to provide a strong high barrier structure and the film thickness ia 220μm.

Main construction materials:

Polyamide PA, higher strength and wear resistance.

Ultra low density polyethylene ULDPE, liquid contact layer with highly clean inner surface



3.2 Physical and chemical properties

3.2.1 Tensile strength test

The tensile strength test is to detect the tensile force required to stretch the material to its breaking point to confirm whether it complies with ASTM D882. This method is applicable to the determination of the tensile strength of plastic films and sheets (thickness should not be greater than 1 mm).

After inspection, the tensile strength at break of the membrane material is 52N/15cm and the elongation at break is 449.2%.

3.3 Barrier performance test

3.3.1 Water vapor, oxygen transmission rate

Oxygen transmission rate testing is the confirmation of the steady state rate at which oxygen will pass through a material. Confirm compliance with ASTM D1434. Gas permeability refers to the volume of gas that permeates a unit area of a sample per unit time at a constant temperature and unit pressure at steady permeation. It is expressed as the volume value under standard temperature and pressure, and the unit is $cm^3/(m^2 \cdot day \cdot bar)$.

Water vapor transmission rate refers to the amount of water vapor permeated by the sample to be tested within a certain period of time under the specified temperature, relative humidity, and certain water vapor pressure difference. The unit is g/(m^2·day). Confirm compliance with ISO 15106-2.

After testing, the oxygen transmission rate is 20.7cm^3/(m^2 · day · bar) and the water vapor transmission rate is 3.3g/(m^2·day).

4. LeRybow® 1/2 liner bag Confirmation

4.1 LeRybow® 1/2 liner bag volume

LeRybow® 1/2 film mianly used on liner bag

Table 1 Volume information of disposable liner bags

type	volume
liner bag	19L-568L

Liner bag can be safely used for the storage of various biopharmaceutical liquids including buffers, culture media, intermediates or raw solutions and can also be used in conjunction with sterile filters. Luer connector, CPC quick connector, Tri-clamp sanitary connector or sterile connector can be used for the inlet and outlet tubing. A variety of sampling methods are provided, such as needless sampler, Luer female connector and medical heparin cap.

4.2 Heat seal strength

4.2.1 Introduction

The purpose of the heat seal strength test is to ensure the firmness of the welding. The welding process of the LeRybow®1/2 liner bag has been verified, and the LeRybow®1/2 film is used to produce a variety of single-use bags.

4.2.2 Method summary

Heat seal the two films according to ASTM F88, cut a 15.0mm ± 0.1 mm wide sample strip from the heat sealing part, clamp the two ends of the sample strip on the two clamps of the testing machine and the distance between the clamps is 50mm, the test speed is 300 mm/min ± 20mm/min, and the maximum load when the sample is separated or broken is read.

4.2.3 Results

The heat seal strength test of the LeRybow®1/2 liner bag showed that they were all greater than 40 N/15 mm.

4.2.4 Conclusion

LeRybow®1/2 liner bag passed the heat seal strength test, all of which were greater than 40N/15 mm.

4.3 Component connection firmness

4.3.1. Introduction

The purpose of the connection firmness test is to confirm the strength of the connection between the tubes and the connectors (including joints, filters, etc.). Usually, cable ties or earrings can be used as fasteners to make the connection more secure. The connection between the pipeline and the joint of the LeRoybow®1/2 liner bag is firm and reliable.

4.3.2. Method summary

Refer to the connection firmness test method GB19335-2003 for static axial tension testing. For joint size ≤ 3.2mm, perform a static axial tension of 30N for 15s; For joint size between 3.2mm and 6.4mm, perform a static axial tension of 50N for 15s; If the joint size is >6.4mm, perform a static axial tension of 80N for 15s.

4.3.3. Results

LeRybow® 1/2 liner bag's tubes and joint connection parts are firm and reliable, joint specification ≤ 3.2mm, each joint should be able to withstand 30N static axial tension for 15s without falling off or breaking; 3.2mm < joint If the specification is ≤6.4mm, each connector can withstand a static axial tension of 50N for 15s without falling off or breaking; if the specification of the joint is larger than 6.4mm, each connector should be able to withstand a static axial tension of 80N for 15s. No detachment or breakage occurred.

4.3.4. Conclusion

The tube and joint of LeRybow®1/2 liner bag are firmly and reliably connected and will not fall off. When using cable ties and earrings as fasteners between the ring interface and the tube, it can play a strengthening role.

4.4 Drop Test

4.4.1 Introduction

The purpose of the drop test is to ensure that the LeRybow® 1/2 bag will not break or leak when dropped from a certain height, the test standard is ASTM D4169-05.

4.4.2 Method summary

Prepare KD 5L, 10L and 20L LeRybow® 1/2 single use bags, 3 bags of each size, after 25~40kGy Gamma irradiation and then fill the marked volume of water at 15~25°C, use a tube clamp secures the tubing. After standing for 24 hours, check the bag for leakage. After confirming that there is no leakage in the test bag, do a drop test according to the standard of ASTM D4169-05, twice horizontally and once vertically, and check whether the bag leaks after each drop test (Table 4).

Table 4 ASTM D4169-05 standard drop test

ASTM D4169-05 drop standard		The article number of the test sample
Sample volume (L)	Drop height (cm)	

【 0，1 】[1]	100	/
【 0，9.1 】	38.1	KD005
【 9.1，18.1 】	33.0	KD010
【 18.1，27.2 】	30.5	KD020
【 27.2，36.3 】	25.4	/
【 36.3，45.4 】	22.9	/
【 45.4，90.7 】	17.8	/
Note: [1] 0~1L refer to YBB0011-2005-2015 Standard: There is no leakage or abnormality in all bags during the test.		

4.4.3 Results

The drop test results of the samples are detailed in Table 5:

Table 5 Sample drop test results

Volume (L)	Process conditions	Drop height (m)	Results
5	25~40kGy irradiation	38.1	no leaks
10	25~40kGy irradiation	33.0	no leaks
20	25~40kGy irradiation	30.5	no leaks

4.4.4 Conclusion

According to the method requirements of ASTM D4169-05 standard, all the samples passed the test and showed no rupture or leakage.

5. Biocompatibility

5.1 In vitro cytotoxicity test

5.1.1 Introduction

According to the ISO 10993-5:2009 chapter, the produced LeRybow® 1/2 liner bag samples were tested to examine their in vitro cytotoxicity. After the test sample extract was cultured with vigorously growing L-929 cells (37°C, 5% CO2) for 24 hours, the cell morphology and cell lysis were observed, and the potential cytotoxicity of the test sample was determined by MTT method.

5.1.2 Results

The cytotoxicity test results are shown in Table 6.

Table 6 Test results

Group	$\bar{x}\pm s$	Cell Viability%	Observe the morphology under the microscope after the extract solution acts on the cells
blank control	0.544±0.021	100.0	0
negative control	0.505±0.022	92.8	0
positive control	0.012±0.016	2.2	4

100% sample extract	0.478±0.009	87.9	0
75% sample extract	0.480±0.018	88.3	0
50% sample extract	0.482±0.020	88.6	0
25% sample extract	0.508±0.027	93.4	0
quality inspection	The average value of blank OD570 ≥ 0.2; the difference between the average value of left (column 2) and right (column 11) and the average value of all blanks is not more than 15%; the test meets the acceptance criteria.		
Conclusion	Under the conditions of this test, the extract of test samples has no potential toxic effect on L-929 cells.		

In summary, the results showed that the cell viability of 100% sample extract was 87.9%. According to the evaluation standard, the cell viability > 70% of the blank group could be judged as having no potential cytotoxicity.

5.1.3 Conclusion

The LeRybow® 1/2 liner bag has no potential toxic effects on cells.

5.2 In viro hemolysis test

5.2.1 Introduction

According to the chapter of ISO 10993-4, the LeRybow®1/2 process bag was subjected to in vitro hemolysis test to check its in vitro hemolysis performance. In vitro tests are used to evaluate whether the test sample has adverse effects on the hemolytic performance. Samples were prepared by the extraction solution method, and diluted blood was added in proportion, placed in a 37°C water bath for 3 hours, taken out and centrifuged, and the supernatant was absorbed and diluted, and the absorbance value was measured with a spectrophotometer (540nm), and the hemolysis rate and hemolysis index were calculated.

5.2.2 Results

The hemolysis rate test results are shown in Table 7.

Table 7 Hemolysis rate test results

method	Group	Parallel number	Is the solution clear/colored after centrifugation		Absorbance	average value	Average hemolysis rate%	hemolysis index%
Extract		DS1	√	N	0.009	0.008	1.48	0.80

	Test samples	DS2	√	N	0.008			
		DS3	√	N	0.007			
	negative control	DN1	√	N	0.007	0.006	0.68	/
		DN2	√	N	0.005			
		DN3	√	N	0.005			
	positive control	DP1	√	R	0.286	0.293	99.20	/
		DP2	√	R	0.293			
		DP3	√	R	0.301			
	blank control	DB1	√	N	0.004	0.004	/	/
		DB2	√	N	0.003			
		DB3	√	N	0.004			
Note: The color of the solution after centrifugation: "N" colorless; "Y" yellow; "Yt" light yellow; "R" red; "RT" light red; "√" clear; Transcript for no clear.								

In summary, under the conditions of this experiment, the hemolysis index of the test sample extract is 0.80%. According to the evaluation standard, when the hemolysis index is between 0-2%, it can be judged as no hemolysis.

5.2.3 Conclusion

The LeRybow® 1/2 linger bag has no hemolytic effects.

5.3 Intradermal reaction test

5.3.1 Introduction

According to the chapter of ISO 10993-10, the test sample extract is directly contacted with the test system to observe its potential intradermal irritation. The samples were extracted with 0.9% sodium chloride injection (polar) and sesame oil (non-polar), respectively, and the sample extract and negative control solution were injected into the back skin of the animal. Then we observed immediately, 24h, 48h and 72h. After these operation, the skin erythema and edema and other reactions were observed. The intradermal irritation reaction of the polar test sample group did not exceed that of the control group, and the average score difference between the sample extract and the negative control solution was 0; The difference between the average score of the control solution was 0.

5.3.2 Results

According to observation, the skin reaction on the test side did not exceed the control skin reaction, and the final score of the skin was 0, see Table 8

Table 8 Observation of Intradermal Reaction Results

Extraction solvent	animal number	group	reaction	24h	48h	72h
0.9% Sodium Chloride Injection	X1501	Test samples	erythema	0	0	0
			edema	0	0	0
		negative control group	erythema	0	0	0
			edema	0	0	0
	X1502	Test samples	erythema	0	0	0
			edema	0	0	0
		negative control group	erythema	0	0	0
			edema	0	0	0
	X1503	Test samples	erythema	0	0	0
			edema	0	0	0
		negative control group	erythema	0	0	0
			edema	0	0	0
sesame oil	X1501	Test samples	erythema	0	0	0
			edema	0	0	0
		negative control group	erythema	0	0	0
			edema	0	0	0
	X1502	Test samples	erythema	0	0	0
			edema	0	0	0
		negative control group	erythema	0	0	0
			edema	0	0	0
	X1503	Test samples	erythema	0	0	0
			edema	0	0	0

		negative control group	erythema	0	0	0
			edema	0	0	0

In conclusion, under the conditions of this test, the extract of test samples did not cause intradermal irritation.

5.3.3 Conclusion

The LeRybow® 1/2 liner bag has excellent biosafety and will not cause intradermal irritation.

5.4 Acute Systemic Toxicity Test

5.4.1 Acute Systemic Toxicity Test - 0.9% Sodium Chloride

5.4.1.1 Introduction

According to the chapter of ISO 10993-11:2017, this test adopts the acute systemic toxicity test method to conduct the acute toxicity test on the test samples. The mice were injected with the extract of the test sample and the negative control solution respectively from the tail vein. During the observation period, the reaction of the animals in the test sample group was the same as that of the animals in the negative control group.

5.4.1.2 Results

It was observed that the reaction of the animals in the test group was the same as that of the animals in the negative control group, as shown in Table 9 and Table 10.

Table 9 Body weight and dosage of mice

Group	animal number	Initial weight (g)	Injection dose (mL)	weight (g)			
				4h	24h	48h	72h
negative control group	J1001	18.6	1.0	18.7	20.1	21.6	22.1
	J1002	17.3	0.9	17.5	19.1	20.7	22.3
	J1003	19.5	1.0	19.6	21.2	22.8	24.3
	J1004	18.4	1.0	18.5	20.0	21.5	23.1
	J1005	17.3	0.9	17.4	18.8	20.2	21.8
Test samples	J2001	18.9	1.0	19.0	20.6	22.1	23.6
	J2002	19.4	1.0	19.5	21.0	22.6	24.1

	J2003	18.2	1.0	18.3	19.7	21.2	22.7
	J2004	17.7	0.9	17.9	19.3	20.9	22.5
	J2005	18.3	1.0	18.4	20.0	21.6	23.1

Table 10 Symptom observation

Group	animal number	symptom			
		4h	24h	48h	72h
negative control group	J1001	—	—	—	—
	J1002	—	—	—	—
	J1003	—	—	—	—
	J1004	—	—	—	—
	J1005	—	—	—	—
Test samples	J2001	—	—	—	—
	J2002	—	—	—	—
	J2003	—	—	—	—
	J2004	—	—	—	—
	J2005	—	—	—	—

Note: - indicates no abnormal symptoms.

In summary, under the conditions of this test, the extract of the test sample did not cause acute toxicity.

5.4.1.3 Conclusion

The LeRybow® 1/2 liner bag has excellent biosafety and will not cause acute toxic reactions.

5.4.2 Acute Systemic Toxicity Test - Sesame Oil

5.4.2.1 Introduction

According to the chapter of ISO 10993-11:2017, this test adopts the acute systemic toxicity test method to conduct the acute toxicity test on the test samples. The mice were respectively injected with the test sample extract and the negative control solution from the abdominal cavity. During the observation period, the reaction of the animals in the test sample group was the same as that of the animals in the negative control group.

5.4.2.2 Results

It was observed that the reaction of the animals in the test group was the same as that of the animals in the negative control group, as shown in Table 11 and Table 12.

Table 11 Body weight and dosage of mice

Group	animal number	Initial weight (g)	Injection dose (mL)	weight (g)			
				4h	24h	48h	72h
negative control group	F1001	18.3	1.0	18.4	20.0	21.5	23.0
	F1002	19.2	1.0	19.3	20.8	21.4	22.8
	F1003	18.7	1.0	18.8	20.4	22.0	23.4
	F1004	17.6	0.9	17.7	19.3	20.8	22.3
	F1005	18.3	1.0	18.5	20.1	21.7	23.3
Test samples	F2001	18.5	1.0	18.7	20.0	21.6	23.1
	F2002	17.6	0.9	17.7	19.1	20.6	22.2
	F2003	19.2	1.0	19.3	20.8	22.4	24.0
	F2004	18.4	1.0	18.5	20.0	21.5	23.2
	F2005	18.0	0.9	18.1	19.6	21.2	22.8

Table 12 Symptom observation

Group	animal number	symptom			
		4h	24h	48h	72h
negative control group	F1001	—	—	—	—
	F1002	—	—	—	—
	F1003	—	—	—	—
	F1004	—	—	—	—
	F1005	—	—	—	—
Test samples	F2001	—	—	—	—
	F2002	—	—	—	—
	F2003	—	—	—	—

	F2004	—	—	—	—
	F2005	—	—	—	—

Note: - indicates no abnormal symptoms.

In summary, under the conditions of this test, the extract of the test sample did not cause acute toxicity.

5.4.2.3 Conclusion

The LeRybow® 1/2 liner bag has excellent biosafety and will not cause acute toxic reactions.

5.5 Skin sensitization test

5.5.1 Skin sensitization test - 0.9% sodium chloride

5.5.1.1 Introduction

According to ISO10993-10, the test sample was extracted with 0.9% sodium chloride injection, and the extract was injected intradermally into 10 guinea pigs, bandaged and tried to induce sensitization. During the recovery period, the sample extract was used for challenge test. Control animals were operated in the same way as the negative control.

5.5.1.2 Results

See Table 13 for the skin reaction results of this test on guinea pigs and the clinical observation and body weight changes of the guinea pigs in this test, and see Table 14 for the positive test results.

Table 13 Allergic skin reactions

Leach solvent	group	animal Numbering	24h after removal of the challenge patch	48h after removal of the stimulus patch	Positive incidence after challenge	Test start weight range (g)	Weight range at the end of the test (g)	Is there any abnormality other than skin reaction
0.9% Sodium Chloride Injection	control group	J1001	0	0	0%	320.5-367.2	480.6-521.7	none
		J1002	0	0				none
		J1003	0	0				none
		J1004	0	0				none
		J1005	0	0				none
	test group	J2001	0	0	0%	317.3-373.7	468.4-531.7	none
		J2002	0	0				none
		J2003	0	0				none
		J2004	0	0				none
		J2005	0	0				none

		J2006	0	0				none
		J2007	0	0				none
		J2008	0	0				none
		J2009	0	0				none
		J2010	0	0				none

Table 14 Positive test results

group	animal number	24h after removal of the challenge patch	48h after removal of the stimulus patch	Positive incidence after challenge	Test start weight range (g)	Weight range at the end of the test (g)	Is there any abnormality other than skin reaction
control group	X1001	0	0	0%	320.7-363.4	464.4-516.5	none
	X1002	0	0				none
	X1003	0	0				none
	X1004	0	0				none
	X1005	0	0				none
test group	X2001	2	2	100%	313.9-359.6	440.9-480.2	none
	X2002	3	2				none
	X2003	3	2				none
	X2004	2	1				none
	X2005	3	2				none

In summary, under the conditions of this test, the extract of the test sample did not cause skin sensitization and the positive rate of sensitization was 0%.

5.5.1.3 Conclusion

The LeRybow® 1/2 liner bag has excellent biosafety and will not cause skin sensitization.

5.5.2 Skin sensitization test - sesame oil

5.5.2.1 Introduction

According to the chapter of ISO10993-10, the test samples were extracted with sesame oil and the extracts were injected intradermally into 10 guinea pigs, bandaged and tried to induce sensitization. During the recovery period, the sample extracts were used for the challenge test. Control animals were operated in the same way as the negative control.

5.5.2.2 Results

See Table 15 for the skin reaction results of this experiment on guinea pigs. The clinical observation and body weight changes of guinea pigs in this test, and Table 16 for the positive test results.

Table 15 Sensitized skin reactions

Extraction solvent	group	animal number	24h after removal of the challenge patch	48h after removal of the stimulus patch	Positive incidence after challenge	Test start weight range (g)	Weight range at the end of the test (g)	Is there any abnormality other than skin reaction
sesame oil	control group	F1001	0	0	0%	318.2-362.0	484.1-527.2	none
		F1002	0	0				none
		F1003	0	0				none
		F1004	0	0				none
		F1005	0	0				none
	test group	F2001	0	0	0%	319.4-359.8	469.7-512.4	none
		F2002	0	0				none
		F2003	0	0				none
		F2004	0	0				none
		F2005	0	0				none
		F2006	0	0				none
		F2007	0	0				none
		F2008	0	0				none
		F2009	0	0				none
		F2010	0	0				none

Table 16 Positive test results

group	animal number	24h after removal of the challenge patch	48h after removal of the stimulus patch	Positive incidence after challenge	Test start weight range (g)	Weight range at the end of the test (g)	Is there any abnormality other than skin reaction
control group	X1001	0	0	0%	320.7-363.4	464.4-516.5	none
	X1002	0	0				none
	X1003	0	0				none
	X1004	0	0				none
	X1005	0	0				none
test group	X2001	2	2	100%	313.9-359.6	440.9-480.2	none
	X2002	3	2				none
	X2003	3	2				none
	X2004	2	1				none

	X2005	3	2				none

In summary, under the conditions of this test, the extract of the test sample did not cause skin sensitization and the positive rate of sensitization was 0%.

5.5.2.3 Conclusion

The LeRybow® 1/2 liner bag has excellent biosafety and will not cause skin sensitization.

5.6 Muscle implantation test

5.5.1 Introduction

The muscle implantation test was carried out with the test sample to evaluate the potential irritation and toxicity of the test substance implanted into the rabbit muscle. By aseptic operation, the test article and the control article were implanted into the muscle tissue of rabbits, meanwile clinical observation was carried out. After 2 weeks, the animals were euthanized, the body was observed grossly and specimens were collected for microscopic observation.

5.5.2 Results

There was no abnormality in clinical observation and naked eye observation at the implantation point. The final stimulation index of the test sample to muscle tissue was 0.6. Under the conditions of this experiment, compared with the control sample, the stimulation level of the test sample to the rabbit muscle tissue is no stimulation.

5.5.3 Conclusion

The LeRybow® 1/2 liner bag has excellent biosafety, without potential irritation and toxicity.

5.7 In Vitro Cytotoxicity Test - USP<87>

According to USP<87>, the produced LeRybow® 1/2 liner bag samples were tested to check their in vitro cytotoxicity. After the test sample extract was cultured with vigorously growing L-929 cells (37°C, 5% CO_2) for 48h, the cell morphology and cell lysis were observed and the cell reactivity was evaluated on a scale of 0-4.

The test results show that under the test conditions, the toxicity of the test sample extract to L-929 cells is 0, and the LeRybow® 1/2 process bag has excellent biological safety and has no potential toxic effects on the cells .

5.8 In Vivo Toxicity Test-USP<88> Class VI

5.8.1 Subcutaneous implantation test

The samples were tested for subcutaneous implantation to evaluate the irritation and toxicity of the test substance after it was implanted subcutaneously in rats.

Under sterile conditions, the prepared test samples and the negative control were respectively implanted subcutaneously in the back of the rats, and the health status of the animals and the clinical observation of the implantation points were observed. After 2 weeks, the animals were painlessly sacrificed and the cysts were observed and recorded. size.

The test results showed that under the conditions of this test, the difference between the test group and the control group in the final average score of cysts was not more than 1.0. The LeRybow® 1/2 liner bag has excellent biological safety, did not cause irritation to the subcutaneous tissue of rats and met the test requirements.

5.8.2 Muscle implantation test

In this experiment, the test sample was tested by intramuscular implantation test method. The test sample and negative control were implanted in the muscle on both sides of the rabbit's back. The size of the implanted capsule was recorded after 2 weeks.

The test results showed that under the conditions of this test, the difference between the test group and the control group in the final average score of cysts was not more than 1.0. The LeRybow® 1/2 liner bag has excellent biosafety and does not cause muscle irritation.

5.8.3 Intradermal reaction test

The test sample was extracted with sodium chloride injection, ethanol-sodium chloride mixture (1:20), polyethylene glycol 400 and sesame oil. The test sample extract and the corresponding control were injected intradermally on both sides of the rabbit's back. All animals were observed for skin reactions by 24h, 48h and 72h after injection.

The test results showed that under the test conditions, within the 72h observation period, the animals in the test sample extract group and the negative control group had the same reaction. The LeRybow®1/2 liner bag has excellent biological safety and the extract does not cause intradermal irritation.

5.8.4 Acute systemic toxicity test

The test samples were extracted with sodium chloride injection, ethanol-sodium chloride mixture (1:20), polyethylene glycol 400 and sesame oil. The mice were injected with four test sample extracts and corresponding controls from the tail vein or abdominal cavity respectively. After the injection, observe the immediate reaction of the mice. Then, observe and record the general state, toxicity performance and number of dead animals in the test group and control group at 24h, 48h and 72h. After that, weigh the body weight of the animals at 72h.

The test results showed that under the test conditions, within the 72h observation period, the animals in the test sample extract group and the negative control group had the same reaction. The LeRybow®1/2 liner bag has excellent biological safety, and the extract did not cause systemic reactions.

6. Particulate matter

6.1 Introduction

The purpose of the insoluble particulate test is to investigate the amount of insoluble particulates in the LeRybow® 1/2 liner bag.

6.2 Method summary

The film is produced on a large scale. In order to avoid excessive liquid filling to cause deviations in test results, the enterprise standard has detailed regulations on the liquid volume (the ratio of sample surface area to extraction medium is 6cm^{2}/mL).

6.3 Results

Test results: less than 50 particles/mL for particles ⩾5 μm, less than 10 particles/mL for particles ⩾10 μm, and less than 25 particle/mL for particles ⩾1 μm.

6.4 Conclusion

According to the test requirements of CHP <0903> and USP <788>, the insoluble particles in the test sample passed all the tests.

7. Bacterial endotoxin

7.1 Introduction

The purpose of the test is to study the level of bacterial endotoxin in the LeRybow®1/2 liner bag. Endotoxin is an exogenous pyrogen that can activate neutrophils to release an endogenous pyrogen that acts on body temperature Regulatory center causes fever.

7.2 Method summary

The film is produced on a large scale. In order to avoid excessive liquid filling to cause deviations in test results, the enterprise standard has detailed regulations on the liquid volume (the ratio of sample surface area to extraction medium is 6 cm^{2}/mL).

7.3 Results

Table 17 Determination results of bacterial endotoxin

Cat. No	Volume (L)	Results
KD050-4C	50	＜0.125 EU/mL
FB113-2C	113	＜0.125 EU/mL
FT113-4C	113	＜0.125 EU/mL
FC200-2C	200	＜0.125 EU/mL
FG500-2C	500	＜0.125 EU/mL
MG500-NXS-40C	500	＜0.125 EU/mL

7.4 Conclusion

The bacterial endotoxin of LeRybow® 1/2 liner bag is less than 0.125 EU/mL, which meets the requirements of Chinese Pharmacopoeia, United States Pharmacopoeia and the internal control standards of LePure.

8. Chemical Compatibility

There are many possible chemical interactions between single-use systems and contact fluids. The pharmaceutical manufacturers should fully consider the consequences of these interactions. If the data on chemical compatibility (including from manufacturers, literature, industry experience, etc.) can be directly cited, pharmaceutical companies can make evaluations based on these chemical compatibility data. ① If other project data in the overall verification project portfolio can be effectively used to assess the risk against chemical compatibility, the industry will usually also conduct reference assessments based on these data. If pharmaceutical companies need to test for chemical compatibility, they can also refer to the relevant test methods of the American Society for Testing and Materials (ASTM) to establish appropriate methods. ②

Notes: ①② China Center for Food and Drug International Exchange: Chapter 3.6 of "Guidelines for Application and Technology of Single-use Systems".

The information in the table below lists the chemical compatibility of ULDPE membranes with common reagents.

Table 18 Chemical compatibility of ULDPE membranes with common reagents

chemical reagent	Chemical compatibility
Acetic Acid 20%	A - Excellent
Ammonium Hydroxide Ammonium Hydroxide 5M	A - Excellent
Barium Sulfate Barium Sulfate	B - Good
Boric Acid	B - Good
Calcium Carbonate	B - Good
Citric acid 1%	A - Excellent
Ethanol	B - Good
Ethyl Acetate Ethyl Acetate	A - Excellent
Glycerin	A - Excellent
Ethylene Glycols	A - Excellent
Heptane	A - Excellent
Hydrochloric Acid 20%	A - Excellent
Hydrogen Peroxide 10%	B - Good
Methanol (Methyl Alcohol)	B - Good
Phosphate 25%	A - Excellent
Propylene Glycol	A - Excellent
Sodium Bicarbonate	A - Excellent

Sodium Chloride 5M	A - Excellent
Sodium Hydroxide 20%	A - Excellent
Sodium Hydroxide 50%	A - Excellent
Sulfuric Acid <10%	A - Excellent
Tween 80 100%	A - Excellent
Sodium hydroxide Sodium hydroxide 1M	A – Excellent
Sodium citrate Sodium citrate 0.2M	A – Excellent
Citric Acid 1M	A – Excellent
Sodium chloride Sodium chloride 1M	A – Excellent
Benzyl alcohol 1%	A – Excellent
Hydrochloric acid 6M	A – Excellent
Tris hydroxymethyl amino methane 1M	A – Excellent
Tris Hydrochloride Tris-HCl 25mM	A – Excellent
Histidine 15mM	A – Excellent
Histidine Hydrochloride 15mM	A – Excellent
Sucrose Sucrose 100mg/mL	A – Excellent
Polysorbate 80 (II) 15%	A – Excellent
Bis(2-hydroxyethyl)imino-trimethylolmethane Bris-HCl 25mM	A – Excellent
Sodium sulfate 125mM	A – Excellent

Note: A – Excellent: excellent, no or negligible interactions
B – Good: good, with minor interactions

9. Gamma irradiation sterilization

9.1 Introduction

Aseptic LeRybow® 1/2 liner bag is a product with high sterility assurance. When LeRybow® 1/2 liner bag is provided in a sterile form, all expected microbial contamination should be reduced to a lowest level before it is sterilized. LeRybow® 1/2 liner bag should be produced in an environment that controls microorganisms. Before sterilization, the number of microorganisms contained is controllable. After the microorganisms are inactivated through the sterilization process, they become sterile products.

9.2 Results

9.2.1 Product Definition

Select typical single-use system components for microbial load testing to obtain initial microbial load data.

According to the definition of ISO11137 regulations, the single-use system series products are classified as a product family during the irradiation sterilization process.

9.2.2 Process definition

Includes precise dosing of radiation, sterility testing methods, validation of bioburden recovery and more. It proved that the pharmaceutical single-use system is suitable for the VD max 25 radiation sterilization dose establishment method and the minimum sterilization dose of the product is 25kGy.

According to the radiation verification results of pharmaceutical single-use system products, during the Gamma sterilization process, the maximum tolerated dose must be more than 1.6 times than the minimum sterilization dose. So, the maximum sterilization dose was set up to 1.6 times for the minimum absorbed dose. The actual sterilization process has a maximum radiation non-uniformity of 40% and the radiation dose range is 25~40kGy.

9.2.3 Dose field distribution test

LePure Biotech entrusts external irradiation processing enterprises to carry out gamma irradiation sterilization processing on products. The radiation dose field distribution test was carried out on the external irradiation device. The test results showed that the radiation dose of the product in each irradiation device was in the range of 25~40kGy.

9.3 Conclusion

According to the above verification process, the results show that the irradiation sterilization dose of the single-use system is set at 25~40kGy, which can reach the sterility assurance level of SAL < 10^{-6}.

10. Shelf life verification

10.1 Introduction

It proves that the LeRybow®1/2 liner bag still maintains good physical, chemical and biological properties after 25~40kGy irradiation sterilization and storage for one year. It proves that the product is in normal use within this validity period and can meet the needs of customers.

Note: The 3-year accelerated and long-term stability investigation and testing work is in progress.

10.2 Method summary

Table 19 Standards for Determination of Product Shelf Life

Test items	Testing Standard
Bag	No creases, scratches, damage, transparent surface, smooth, uniform color, no perforation, odor, foreign matter, no foreign matter visible to the naked eye; the heat-sealed part of the bag body should be flat and free of voids
Tubing	The material has uniform color, no impurities, no damage, no burrs, no air bubbles larger than 1/4 of the wall thickness and no continuous air bubbles larger than 1cm
Ports/Joint	There is no foreign matter visible to the naked eye at the joint between the tube and the bag
Retention clip	It can cut off the current, and it can be switched ten times without damage
Bacterial endotoxin tests	Need to be less than 0.125EU/mL
Insoluble particles tests	The value should be less than the standard specified in the test sample, the particle diameter ⩾ 5μm, 10μm and 25μm should not exceed 50, 10, and 1 particles respectively.
Visible particulate	No more than 3 microscopic visible foreign matters per 50mL of test solution.
Integrity check	The tested bag filled with the marked volume is left for 24hours without leakage
Connection firmness	Must be greater than the tension specified in the standard
Heat sealing strength	The strength of each heat sealing part is not less than 50N/15mm
Chemical test	pH value, UV absorption, heavy metals, reducing substances, metal ions
biological test	Cytotoxicity and Sterility Testing

10.3 Results

LeRybow® 1/2 liner bag body, tube, port, joint, retaining clip, bacterial endotoxin, insoluble particle detection, visible foreign matter, integrity detection, connection firmness, heat seal strength, chemical test, biological test meet the testing standards.

10.4 Conclusion

LeRybow® 1/2 liner bag are produced in Class C or Class C+A clean room and finally sterilized by irradiation to ensure the sterility of the product. Under normal temperature and dry storage conditions, the validity period of the product is 24 months from the date of production.

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