SARTORIUS

Technical Report of Flexsafe® 3D Bags Qualification

For Liquid Shipping According To ASTM D4169

Technical Note

Scope – This technical report describes the qualification of our liquid shipping solutions for Flexsafe® 3D bags from 100L to 500L in order to provide contained and robust liquid shipping. The Flexsafe® shipping systems have been extensively tested against the most stringent international standards ASTM D4169. Filled Flexsafe® 3D Bags have been qualified with Sartorius Stedim's shipping Palletank via a 4-step qualification program for liquid transportation.

Executive Summary

Sartorius Stedim Biotech has qualified liquid shipping for Flexsafe® bags in the specified shipping Palletank in order to support end-users for shipping biotech fluids, like media, buffer, intermediates, drug substances and drug product. The liquid shipping qualification is designed to answer the new challenges associated with the growing adoption of single-use technologies in more critical process steps of cGMP commercial productions and the increased need of transportation of valuable liquids all around the world. This technical report provides extensive data for liquid shipping in single-use systems based on a 4-step method and all qualification tests performed according to ASTM D4169-14.

Understanding the product lifecycle and associated risks are pre-requisites to establish the suitable qualification testing approach. For truck or international transportation conditions, the shipping system must withstand various mechanical shocks and vibration levels to maintain container closure. Preliminary testing under real conditions and in laboratory allowed to evaluate the strength of the shipping system.

Our Flexsafe® 3D bags from 100L to 500L in the shipping Palletank are qualified for liquid shipping at nominal volume under the international norm ASTM D4169-14 level II for cycle 12 & 14 at 4°C (39.2°F) and at 40°C (104°F).

1. Real shipping conditions
Analysis of acceleration measured on filled Flexsafe® shipping system during real transportation (including handling, truck and airplaine shipments).

2. Laboratory tests with norms
Analysis of acceleration measured on filled Flexsafe® shipping system during testing in laboratory according to ASTM D4169-2014 at different severity levels and ISTA 3E.

3. Qualification protocol determination
Real transport cycle analysis and comparison with laboratory testing results in order to choose the right setting parameters for Flexsafe® bags qualification with ASTM D4169-2014.

4. System qualification
Filled Flexsafe® 3D bag qualification according to ASTM D4169-2014 assurance level II for cycle 12 & 14 at 4°C (39.2°F) and 40°C (104°F).

Qualification Approach

Shipping systems must be qualified for their intended use through proper design and testing in consultation with a packaging engineer. ISTA1 and ASTM D41692 are the known standard norms for testing. This technical report provides the rational, testing program and results for filled Flexsafe® 3D bags.

In order to qualify the system with the more relevant testing parameters and provide reliably contained liquid shipping systems, several preliminary tests have been performed before the final qualification testing program, as described below:

1. Real conditions analysis of the acceleration measured on filled Flexsafe® shipping systems have been performed for a variety of truck, airplane and handling sequences.
2. Tests have been performed to characterize the shipping system vibratory solicitations at different configurations (example: transfer function for one or two systems per pallet). Same measurements have been performed on filled Flexsafe® shipping systems when submitted in laboratory to all the different levels of severity for the ASTM D4169-14 norm and the ISTA 3E test sequence.
3. Data gathered during tests 1 & 2 have been analyzed and compared in order to choose the appropriate qualification testing sequence that would cover all the real conditions shipment with a comfortable very high impact level. The ASTM D4169 level II testing sequence was the more relevant testing program covering truck, airplane shipping conditions with high buffer margin. In addition many pharmaceutical or biotechnological products are temperature sensitive and required specific precaution during storage and transportation. Transport qualification tests have to be done to bracket common operating temperature ranges.
4. Flexsafe® 3D bags for 100L , 200L and 500L in the shipping Palletank have been tested according to ASTM D4169-14 level II with the distribution cycle 12 &14 at 4°C (39.2°F) and at 40°C (104°F) with the worst case configuration.

This technical report3 provides the materials, methods, testing program and results for the Flexsafe® 3D liquid shipping solutions.

Technical and Regulatory Background

Regulatory agencies like FDA4, EMA5 or EU6 emphasize the need for end-user to ensure that their drug processes produce consistent and reproducible results which meet the quality standard of the drug product. Validation is “Establishing documented evidence that provides a high degree of assurance that a specific process” including shipping “will consistently produce a product meeting its pre-determined specifications and quality attributes” (FDA4). A properly designed system will provide a high degree of assurance that every process step, including shipping has been properly evaluated before its implementation.

In the biopharmaceutical industry, qualification and validation are intended to demonstrate that the manufacturing process provides the desired level of compliance of the product and specifically its activity, sterility and potency. Qualification of a shipping system and equipment is part of the process validation.

According to the PDA technical report N°667, "Shipping systems must be qualified for their intended use through proper design and testing in consultation with a packaging engineer. The transportation routes must be defined for international shipment. A risk assessment for vibration, handling, delays and seasonal variation should be established.

International standards description

ASTM or ISTA are well-known standards for shipping systems. They are aimed to compare or evaluate the effectiveness of protective packaging and | or a packaged-product's ability to withstand the hazards of distribution. Table 1 briefly describes the main features of ASTM and ISTA standards.

ASTM D4169 – Standard practice for performance testing of shipping containers and systems	**ISTA 3-Series: General simulation performance tests**
General simulation tests covering a range of package types and distribution scenarios. The user must choose from tests, alternatives, intensities, sequences and specific procedures based on packaged-product and distribution characteristics. Applicable across broad sets of circumstances, such as a variety of vehicle types and routes, airplane, boat, rail, or a varying number of handling exposures. Tests are carried out sequentially on the same package. 18 Distribution Cycles (DC): DC should be chosen close to the projected distribution, like for example: – Preconditioning and conditioning – Handling – Shock (Horizontal impact, Rotational flat drop and Edge drop) – Vibration truck – Low pressure – Air vibration – Compression (optional) Three levels of severity (I, II, III) are described in the ASTM D 4169	Designed to provide a laboratory simulation of the damageproducing motions, forces, conditions, and sequences of transport environments. Applicable across broad sets of circumstances, such as a variety of vehicle types and routes, or a varying number of handling exposures. For example: 3E & 3H tests consist of 7 to 15 individual tests that are carried out sequentially on the same package. The test simulates the handling and transit required in a road distribution network and covers truck transport only. It is composed of sequences including for example: – Preconditioning and conditioning – Shock (Horizontal impact, Rotational flat drop and Edge drop) – Vibration only truck – Compression (optional)

Table 1: ASTM and ISTA standards

The definition of the system to be tested and the testing program need to be carefully assessed and justified. The selection of the right testing parameters and the tested samples' configurations can be done only by knowing the transportation cycles and the type of impact perceived by the load during the transportation, thus requiring preliminary testing and analysis.

International standards selection

The protocol of test needs to correlate to the projected life cycle phase of the shipped unit. As a first step, knowledge of shipped product and the type of transportation (mean and sequences) are key to understand the shipping cycle and provide the adequate buffer margin during qualification testing program.

A typical distribution sequence between two plants in the biotech industry is described by the following drawing:

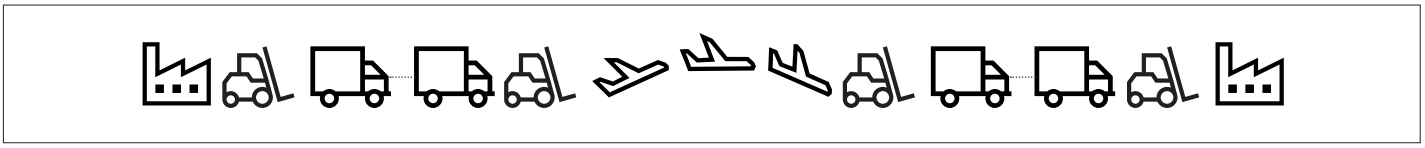


Figure 1: typical distribution cycle

The distribution cycle DC 12 of ASTM D4169 is representative of the typical shipment shown in the figure above for loads >68.1 kg (150 lb) or unitized shipment. DC12 includes 6 modular test programs adapted to simulate each segment of the projected distribution (Table 2) with impact (horizontal impact, rotational flat drop and edge drop), low pressure (representative of shipment by plane or high altitude) and vibration tests.

Test name	Short description	Tests example for unitized load
Schedule A: Handling	Manual & mechanical handling determines the ability to withstand loading, unloading, stacking, sorting, palletizing, ... operations	Example: Mechanical handling: ▪ Fork lift handling – rotational flat drop test ASTM D6179 ▪ Side impact test – horizontal shock on sides of the shipping unit ASTM D880 ▪ Truck handling – ASTM D6055
Schedule D: Stacked vibration	Stacked vibration determines the ability to withstand the dynamic compression forces resulting from vehicle stacking	Example: ▪ stacked load to be calculated and stacked system submitted to random vibration profile – ASTM D4728
Schedule I: Low pressure (High altitude) Hazard	Test to anticipate the impact of a reduced pressure when the system is transported via aircraft	Usual recommended test conditions: ASTM D6653 at a pressure equivalent to 4,267 m (14,000 ft) for a period of 60 min
Schedule E: Vehicle vibration	Vibration test submitting the load to a random vibratory profile	Truck vibratory profile ASTM D4728, Method A Air vibratory profile ASTM D4728, Method A
Schedule J: Concentrated impact	This test provides a simulation of anticipated low level concentrated impacts as received by packages during sorting operations and in transit	Test method described in ASTM D6344
Schedule A: Handling	Manual & mechanical handling determines the ability to withstand loading, unloading, stacking, sorting, palletizing, ... operations	Example: Mechanical handling: ▪ Fork lift handling – rotational flat drop test ASTM D6179 ▪ Side impact test – horizontal shock on sides of the shipping unit ASTM D880 ▪ Truck handling – ASTM D6055

Table 2: Distribution Cycle N°12 of ASTM D4169

The choice of the testing program needs to fit the type of tested load and the parameters needs to be determined with a rational based on the shipping conditions and | or experience and packaging expertise.

The next step is to define the severity of testing (level and duration). For the Flexsafe® shipping system qualification, the level of severity has been defined according to a desired buffer margin chosen over the real shipping tests impacts. The combination between real shipping tests and laboratory tests for the system characterization with ASTM D4169 and ISTA3E provided the relevant data helping to select the appropriate qualification method, parameters and acceptance level.

Materials and Methods

1. Flexsafe® 3D Bags 100L, 200L and 500L with shipping Palletank

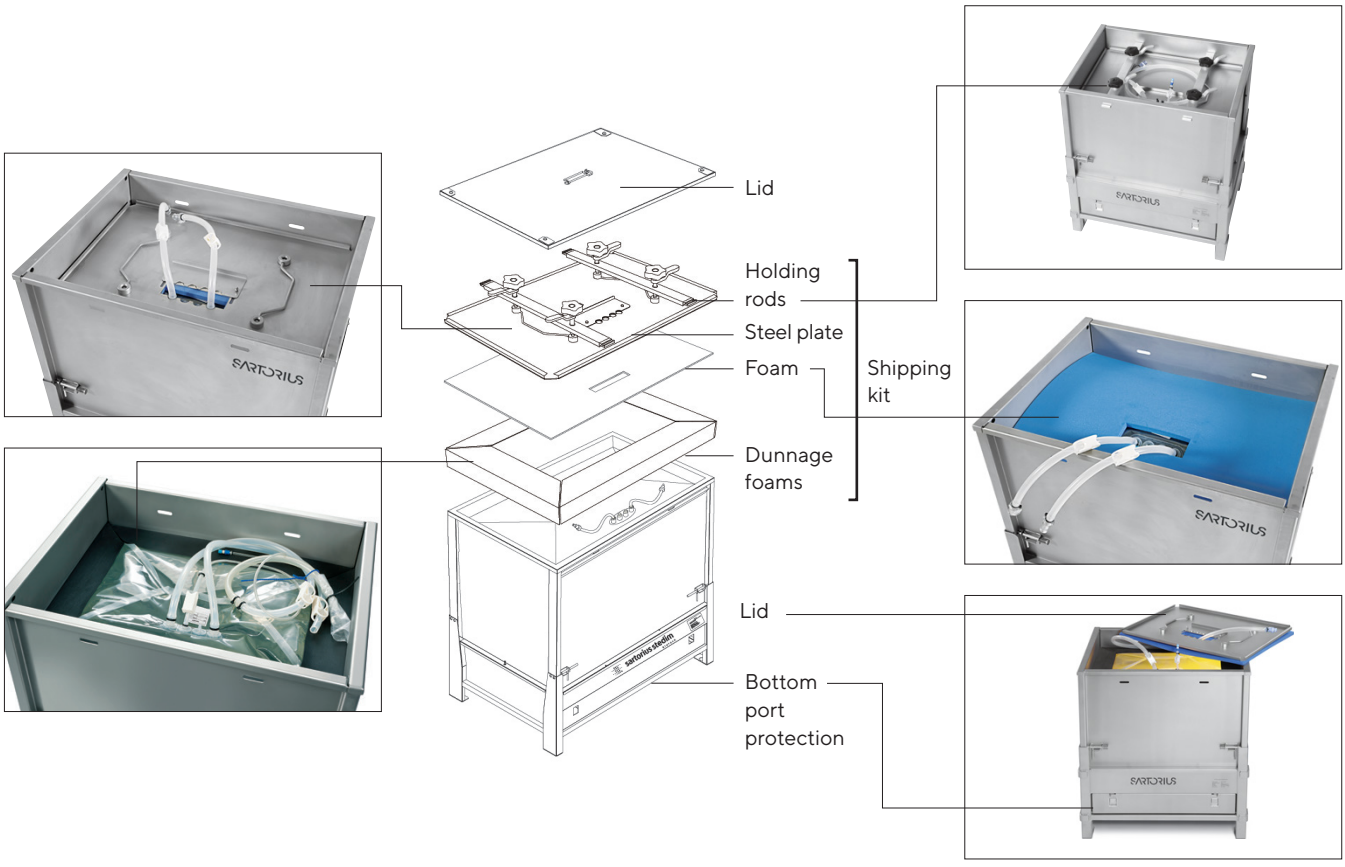


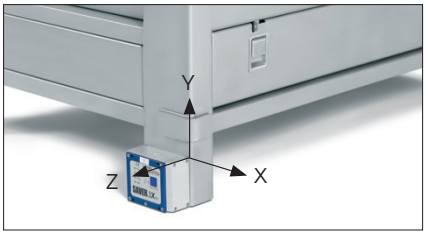
Figure 2: Palletank shipping system

Data recording:
Filled Flexsafe® 3D Bags in the shipping Palletank have been equipped with a data logger which measures acceleration, temperature and humidity.

The acceleration [$m.s^{-2}$] of the system is the raw data further analyzed for all testing conditions.
These conditions are defined in the table 3 below:

Conditions	Signal denomination	Data recording frequency
Real transportation	Continuous signal	30s within 2 or 5 minutes
	High signal	Each time acceleration > 0.3 g
Laboratory	ASTM or ISTA signal	Entire signal

Table 3: Recorded data origin



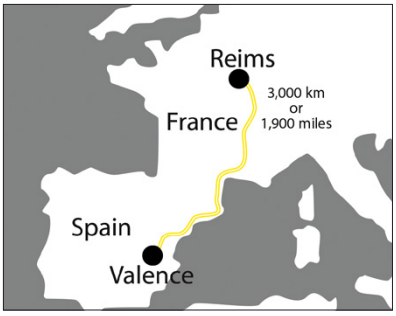
The data logger is fixed with a rigid device on the foot of the Palletank to ensure that the measured answer is as close as possible of the solicitations.

1. Real Shipping Conditions

1. Real shipping conditions
Filled Flexsafe® shipping system analysis during real transportation.

In order to understand what happens during the most used transportation means for biopharmaceutical fluids, filled Flexsafe® 3D Bags in their shipping Palletank have been equipped with a data logger and shipped under two real transportation cycles:

Cycle 1 – European transport:
this shipment has been performed in Europe including several phases of handling and truck. It represents a transportation of more than 3,000 kms | ~1,900 miles.



Cycle 2 – International transport:
this shipment has been performed from Europe up to India including several phases of handling, truck, tarmac and airplane. It represents a transportation of more than 30,000 kms | ~19,000 miles including 400 km of severe truck driving.

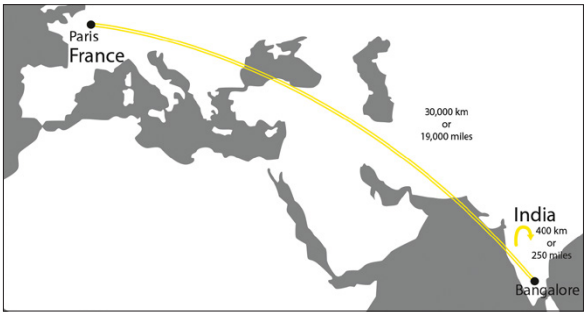


Figure 3: Real transportation cycles

For cycles 1 & 2, two Flexsafe® 3D bags per volume (100L, 200L & 500L) filled with water are installed in their shipping Palletank. During both cycles, the data are recorded for quantification of vibrations (intensity, duration, shakes) and level of shocks perceived by the load during the transportation for different configurations.

2. Laboratory ASTM D4169 and ISTA3E Tests

2. Laboratory tests with norms
Laboratory testing according to ASTM D4169-2014 and ISTA 3E.

The knowledge of the behavior of the systems when tested under the normative protocol is a key factor. Water filled Flexsafe® 3D Bags (1 bag per volume: 100L, 200L & 500L) in their shipping Palletank have been equipped with a data logger and tested according to ASTM D4169 level I, II and III and ISTA 3E. Transportation configurations (with and without a pallet between the vibratory table and the Palletank) have been tested to define the worst case for the system qualification testing.

The laboratory measurements started with the shipping system behavior characterization, performed by injecting on the vibratory table a “flat” spectral density. The output is a transfer function with natural frequency of the system.

3. Comparison of 1 & 2

3. Qualification Protocol Determination
Real transport cycle analysis and comparison with laboratory testing.

All data measured in both previous test sequences have been analyzed and compared in order to define the most relevant qualification testing protocol to apply during Flexsafe® qualification.

The approach is summarized below:

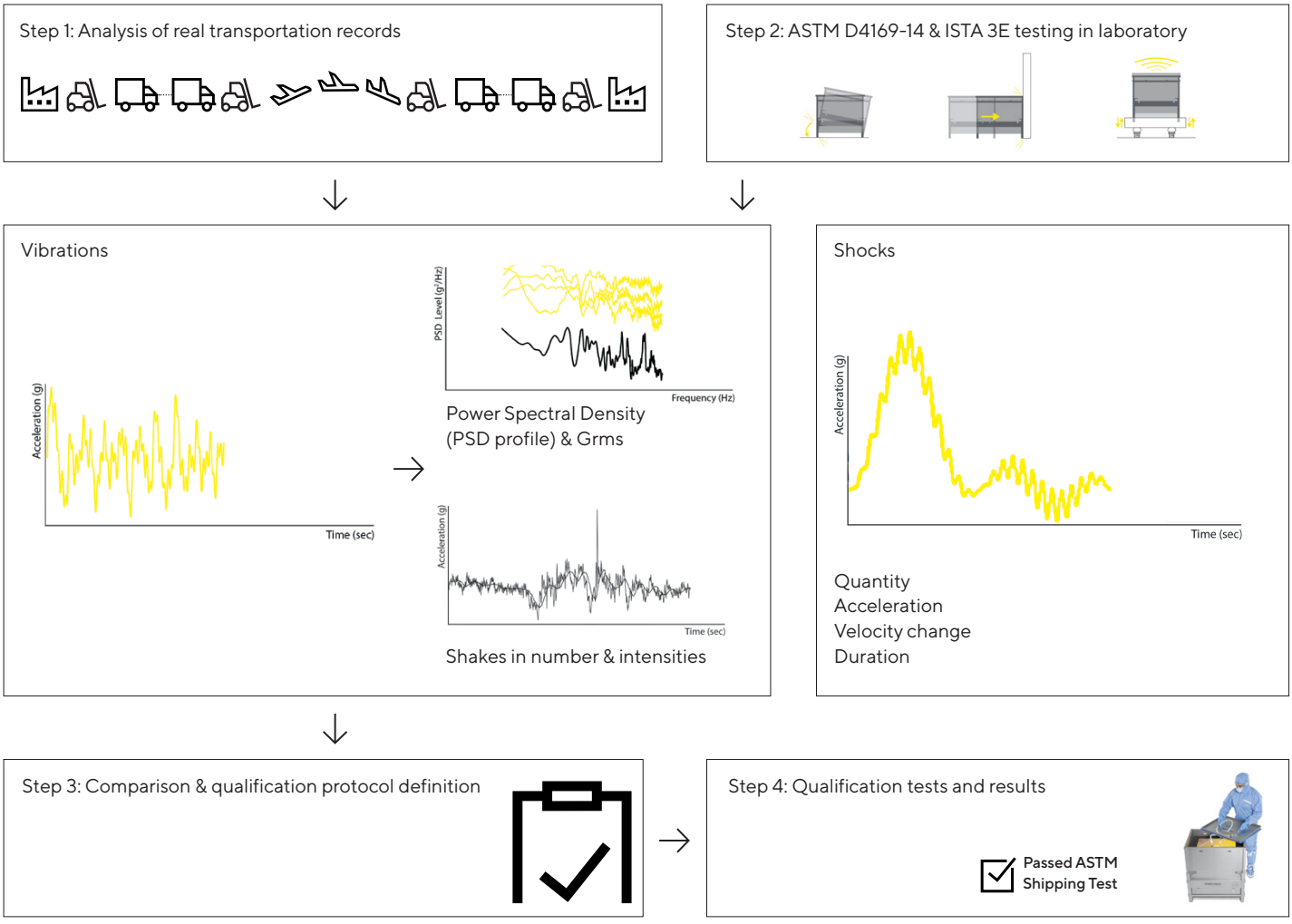


Figure 4: Flexsafe® shipping system qualification principles

Comparison of real transportation measurements and measurements performed during ASTM D4169 and ISTA3E in addition of typical transportation cycle analysis allow to define an adequate testing protocol and the worst case configurations to be tested.

4. Qualification of Flexsafe® 3D Bags 100L to 500L in Shipping Palletank

4. System qualification
Filled Flexsafe® 3D bag qualification according to
ASTM D4169-2014 assurance level II for cycle 12 & 14
at 4°C (39.2°F) and 40°C (104°F).

Flexsafe® 3D bags from 100L, 200L and 500L after gamma irradiation at 50 KGy (worst case) are installed in their shipping Palletank with the shipping kit after being filled at their nominal volume with water. Four bags per volume taken from two different batches are tested under the testing protocol defined previously. Qualification tests have been performed at 4°C (39.2°F) and 40°C (104°F) with previously defined worst case configurations.

Test sequence		Test reference based on ASTM D4169-14 – Assurance level II
1	Pre-conditioning	4°C (39.2°F) or 40°C (104°F) during 72 hours
2	Mechanical handling	SCHEDULE A
2.1	Truck handling	ASTM D6055 Method A | 5 cycles round trip
2.2	Horizontal impact	ASTM D880 Method B | each side 1 shock (total 4)
2.3	Rotational flat drop test	ASTM D6179 Method C | 2 drop tests (short and long edge)
3	Vehicle vibration	SCHEDULE E
3.1	Truck spectrum	ASTM D4728 Method A – 90 minutes truck spectrum
3.2	Air spectrum	ASTM D4728 Method A – 90 minutes airplane spectrum
4	Low pressure	SCHEDULE I
5	Mechanical handling	SCHEDULE A
5.1	Truck handling	ASTM D6055 Method A | 5 cycles round trip
5.2	Horizontal impact	ASTM D880 Method B | each side 1 shock (total 4)
5.3	Rotational flat drop test	ASTM D6179 Method C | 2 drop tests (short and long edge)
6	Warehouse stacking	SCHEDULE B ASTM D642 – 3s

Table 4: Chosen ASTM D4169 testing program for Flexsafe® qualification

After the ASTM tests cycles, the systems are inspected for the absence of damage and the bags are inspected for the absence of leakage by visual inspection and dye penetration test. Acceptation criteria after the tests:
- Visual inspection during test – no leak, no damage on the system
- Visual inspection of the emptied bag – no leak
- Dye penetration test on emptied bag – no leak

Tests Results

1. Real Shipping Conditions

1. Real shipping conditions

Filled Flexsafe® shipping system analysis during real transportation.

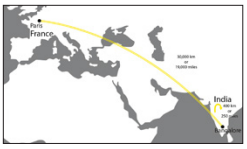
Cycle	Real condition		Description	Duration (HH:min)	Mileage (km)	Tested systems
1		Handling 1	Loading | Unloading	Minimum 3 handling phases		
		Europe 1 (Truck)	Highway France	16:15	1,410	200L × 1 500L × 2
			Road France	04:29	350	–
			Highway France	15:49	1,420	No pallet
2		Handling 2	Loading | Unloading	Minimum 10 handling phases		
		Europe 2 (Trucks)	Highway	14:17	~1,100*	100L × 1 200L × 2 500L × 2
		India 2 (Trucks)	Mixed roads	06:35	~400*	
		Tarmac	Tarmac	01:09	/	–
		Plane	Cargo area	17:20	/	One per pallet

Table 5: Real shipping conditions. Note that each travel cycle was a round trip.
* no GPS measurement

During both real shipping travels, data was recorded for further quantification in number and intensity for vibrations, shakes and shocks seen by the load during the transportation for 100L, 200L and 500L.

1.1 Principle for analyzing recorded data

Packaging experts use the frequency analysis for each travel phase for more readable data with the PSD graph (Power Spectral Density)

Definition

PSD = Power Spectral Density. It is the frequential decomposition of a random vibratory signal. The PSD is expressed in g^2/Hz according to the frequency Hz (figure 6).

- The Vibratory level "Continuous" (in grey) is representative of the felt vibrations during the full transportation time. It is measured in taking up a vibration sample by regular time interval (every 2 or 5 minutes) and in calculating the samples' average of a particular phase. This indicator is representative of the continuous background of vibration.
- The High vibration level "High" (in orange) represents the maximum of recorded vibrations. It is the average of exceptional events (trigger >0.3 g) met during a phase.

1.2 Example of data recording for 100L

For each high vibratory excitation (event >0.3 g), an event is recorded.

The following chronogram shows the apparition density of vibratory events for the two 100 L Flexsafe® Bag in Palletank. This density gives the high events number by time unit of 1 hour.

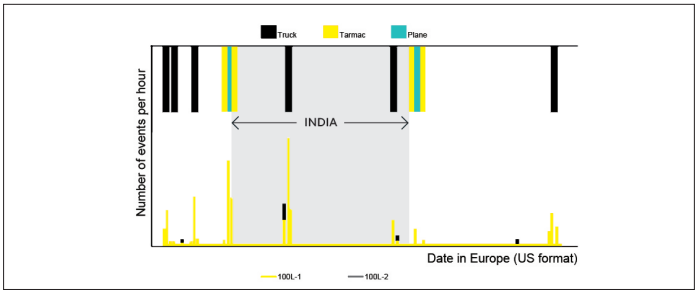


Figure 5: Graph with number of events recorded per time unit

In this example, we note that the number of vibratory event per hour varies greatly during the distribution cycle. The truck India phase is intense. (1) and the first tarmac phases are intense. (2). The PSD graph for the 100L truck phase example provides a relevant way of evaluating the vibration level per frequency during the travel phase.

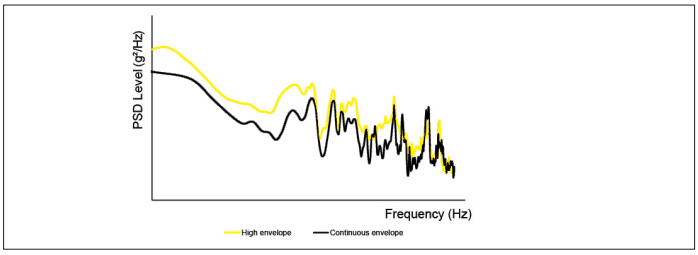


Figure 6: PSD graph for 100L real transportation

Results summary

The amount of data7 is very important; the table below provides only a selection of some curves and some relevant data. (Table 6)

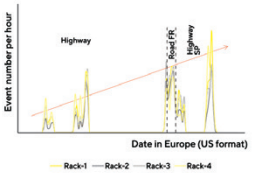
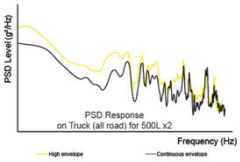
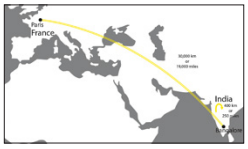
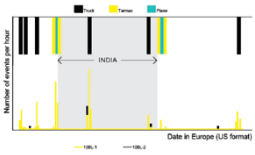
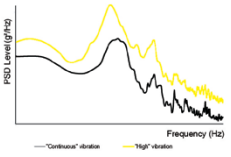
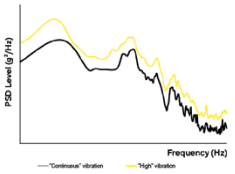
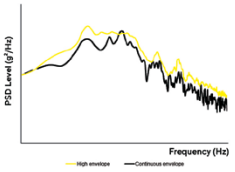
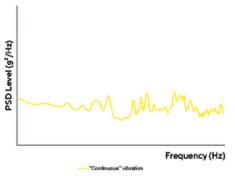
Cycle	Real condition		Tested systems	Chronogram of "High" events	Vibration per travel phase		
1		Handling 1			PSD curves	Grms*	Shakes
		Europe 1 (Truck)	200L ×1 500L ×2 – No pallet	Events number per hour 	Global PSD 	200L 0.065 500L 0.084	200L Nber: 331 shakes Max: 2.9 g 500L Nber: 73 shakes Max: 1.9 g
		Handling 2			PSD curves	Grms*	Shakes
2		Europe 2 (Truck)	100L ×2 200L ×2 500L ×2 – One per Pallet	Events number per hour 		100L 0.094	100L Nber: 1,781 to 2,721 Max: 7.3 to 9.8
		India 2 (Truck)		Only 100L system is displayed as an example in the cycle 2		100L 0.187	
		Tarmac				100L 0.216	100L Nber: 1,206 to 1,727 Max: 9.7 to 10.8
		Plane			 No high event recorded	100L 0.032	None

Table 6: Summary of results analyzed from the recorded real shipping data.
* Grms for "continuous" recording

Results summary
Table 6 continued

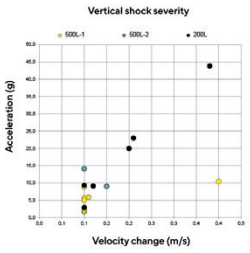
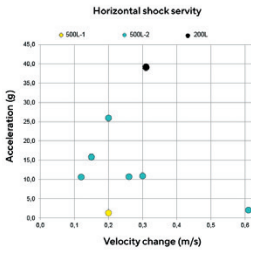
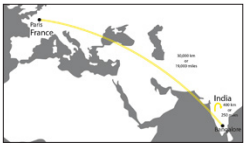
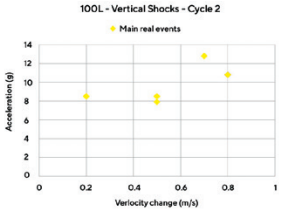
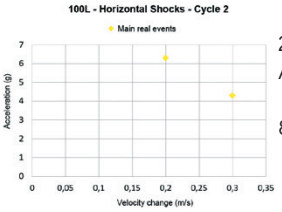
Cycle	Real condition		Tested systems	Shocks – exceptional events analysis		Climatic chronogram
1		Handling 1		Vertical shocks	Horizontal shocks	
		Europe 1 (Truck)	200L ×1 500L ×2 – No pallet	Severity diagram*  Maximum acceleration values from 1 to 45 g	Severity diagram*  Maximum acceleration values from 1 to 40 g	0.5 to 1.5°C
2		Handling 2		Vertical shocks	Horizontal shocks	
		Europe 2 (Truck) India 2 (Truck) Tarmac Plane	100L ×2 200L ×2 500L ×2 – One per Pallet	Severity diagram*  Maximum acceleration values from 8 to 13 g. The pallet provides an important attenuation effect	Severity diagram*  Maximum acceleration values from 4 to 6 g. The pallet provides an important attenuation effect	10 to 35°C Average: 21°C 20 to 80% RH Average: 50% 800 to 1,000 mbar

Table 6: Summary of results analyzed from the recorded real shipping data.
* severity diagram: acceleration (g) in function of velocity change (m/s)

All the recorded data will be used for the chapter 3, when comparing the real shipping results to the laboratory tests.

Conclusion
These records provide extensive data in real shipping in several types of conditions, systems and configurations. All the Flexsafe® Bags 100L, 200L and 500L in their shipping Palletank remained integer during the all real shipping travels.

2. Laboratory ASTM D4169 and ISTA 3E Tests

2. Laboratory tests with norms

Laboratory according to ASTM D4169-2014 and ISTA 3E.

The aim of these test is to understand and characterize the impact of the tests according to ASTM D4169 all levels and ISTA 3E on Flexsafe® filled bags when used in different configurations (with or without a pallet)

Testing table

Shipping solution configurations		"Flat" spectrum in laboratory	ASTM D4169 level I	ASTM D4169 level II	ASTM D4169 level III	ISTA 3E
100L	Without pallet	Transfer function & natural frequencies of each system & each configuration	Vehicle vibration: truck and air profiles (PSD, Grms, shakes) Shocks: horizontal and rotational flat drop test			
	One per pallet					
	Two per pallet					
200L	Without pallet					
	One per pallet					
	Two per pallet					
500L	Without pallet					
	One per pallet					

Table 7: Testing program for ASTM D4169 and ISTA 3E per system and per configuration

2.1 Mechanical behavior: Shipping system vibratory characterization

The laboratory measurements started with the shipping system behavior characterization, performed by injecting on the vibratory table a "flat" spectral density. The output is a transfer function with natural frequencies of the system. The test consists in submitting the shipping system (bag and container) to a panel of multifrequential vibrations. The excitation vibration is shown by its spectral density (PSD excitation). In laboratory condition, "flat" spectrum (multi frequential) from 2 Hz up to 200Hz provide ampli tudes which allows to stay in a pure vibratory mode (no shakes generated). This "flat" spectrum is applied on the shipping system for several transport configurations (with and without pallet, 2 containers per pallet when possible). The input signal is described in the following graph:

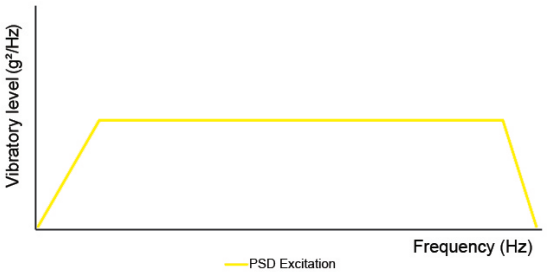
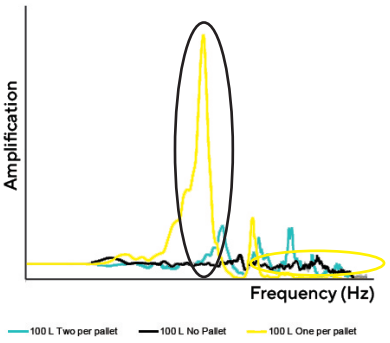


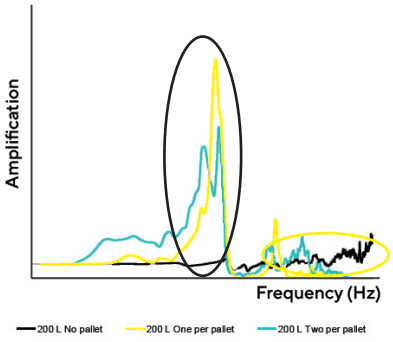
Figure 7: "Flat" spectrum excitation

The output acceleration is then measured and the transfer function of the system is obtained for each frequency bandwiths by calculating the ratio between the response and the excitation intensity according to frequency. There is an amplification when this coefficient is above 1, and an attenuation when it is below 1. This characterization has been performed for 100L, 200L and 500L Flexsafe® filled bags in shipping Palletank in different configurations:

- Without pallet: Palletank directly in contact on the vibratory table
- One on pallet:
  One Palletank on one pallet (middle) for 100L, 200L and 500L
- Two on pallet:
  Two Palletank on one pallet for 100L and 200L.

The associated transfer function has been analyzed:





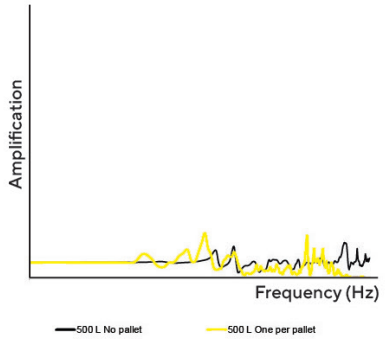


Figure 8: Transfer function for each system (100L, 200L and 500L)

Conclusion for pure vibratory solicitations

Generally, pallet is amplifying low frequencies below and attenuating high frequencies.

For low frequencies, 100L and 200L configurations with one container per pallet represent the worst case (highest amplification) compared to configurations without any pallet. Above 20Hz, this configuration shows a damping effect (attenuation).

For the 500L Palletank, there is no significant difference between no pallet and one system per pallet, except for frequencies above 100Hz where the pallet has a damping effect.

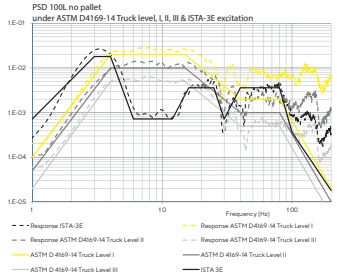
2.2 ASTM D4169 & ISTA 3E Measurements

ASTM D4169 and ISTA 3E tests are testing programs evaluating the impact of vibration (PSD, Grms & shakes) and shocks for different parameter settings. The aim of these measurements is to record the behavior of each shipping system when submitted to the testing programs.

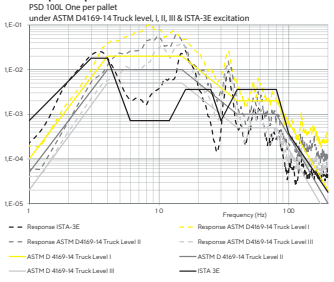
2.2.1 Analysis between ASTM D4169 and ISTA 3E for vehicle vibration – truck & air

Configurations	Vehicle vibration
	1. Schedule E – ASTM D4278 method A – Truck and air 2. ISTA 3E – Truck Results summary for vibrations (PSD graph), accelerations (Grms graph) and number of shakes
100L ▪ without pallet ▪ one per pallet ▪ two per pallet	PSD graph for 100L for all configurations

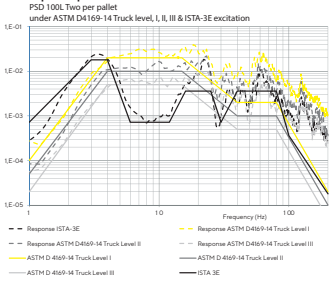
Truck: Without pallet



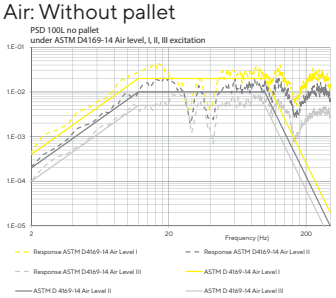
One per pallet



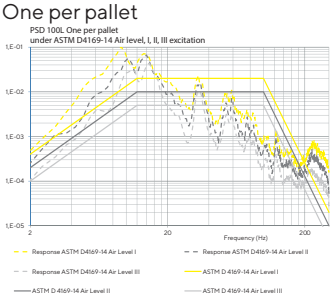
Two per pallet



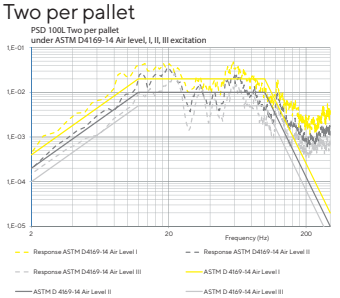
Air: Without pallet



One per pallet

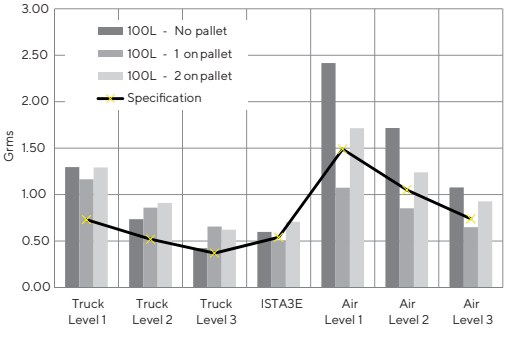


Two per pallet



Grms and shakes analysis for 100L for all configurations

100L



Shakes during 90 min - 100L

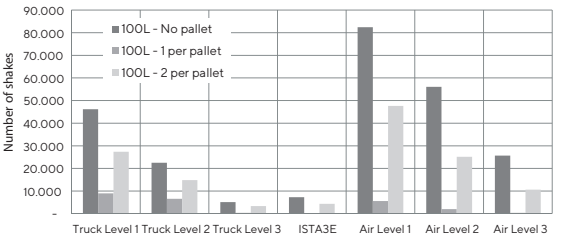


Table 8: Results summary example for 100L system for vibrations (PSD graph), accelerations (Grms graph) and number of shakes during 90 min.

Depending on the configurations (with or without pallet), the impact of the tests are significatively different, especially for their severity. These data is used for comparing with real shipment and defining the worst case configurations for the qualification testing program.

Comparison of the acceleration when using ISTA 3E and ASTM D4169 level II – Example of shakes

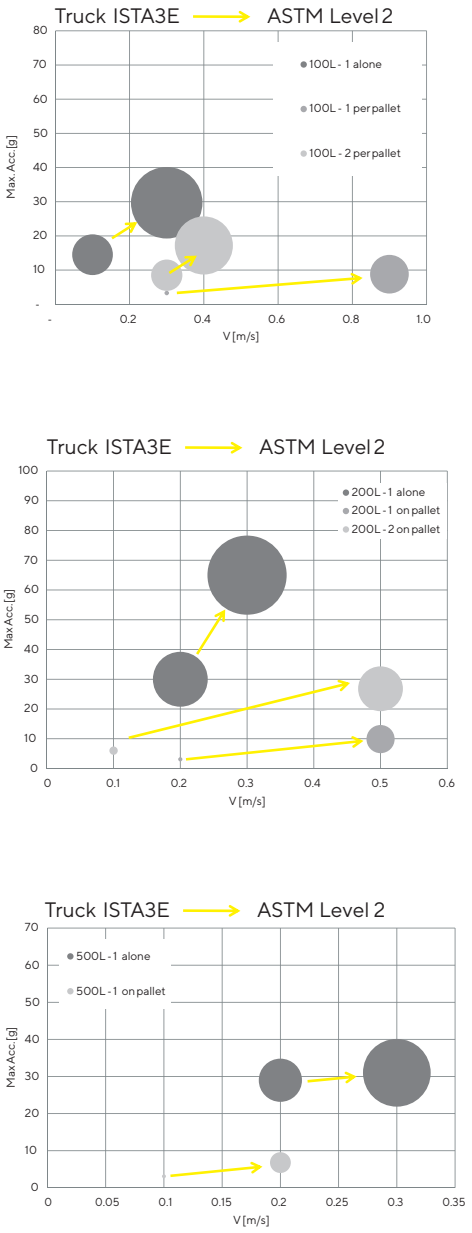


Figure 9: Severity graphs comparing ISTA 3E 90 min and ASTM D4169 level II 90 min

The comparison is made on shakes as a indicator of vibration intensity. As a conclusion, ISTA 3E is always less severe than ASTM-D4169 level II.

3. Comparison Between Real and Laboratory ASTM D4169 & ISTA Results

3. Qualification protocol determination
Real transport cycle analysis and comparison with laboratory testing.

Comparison of real transportation measurements and measurements performed during ASTM D4169 and ISTA3E in addition of typical transportation cycle analysis allow to define an adequate testing protocol. The comparison has been performed on the results for vehicle vibration in terms of PSD, Grms and shakes and for horizontal and vertical shocks.

3.1 Results and analysis principles
An enormous amount of data has been analyzed for this comparison. This is the key step providing the rational and scientific justification for the choice of the adequate qualification parameters and the choice of the Flexsafe® systems configuration. During real transport measurements and laboratory testing according to the ASTM and ISTA norms, vibrations and shakes were recorded in order to assess the severity of vibrations. Vibration severity is characterized by PSD curves, Grms level, duration of solicitations and number of shakes generated for each vibration phase: Truck, Airplane and Tarmac.

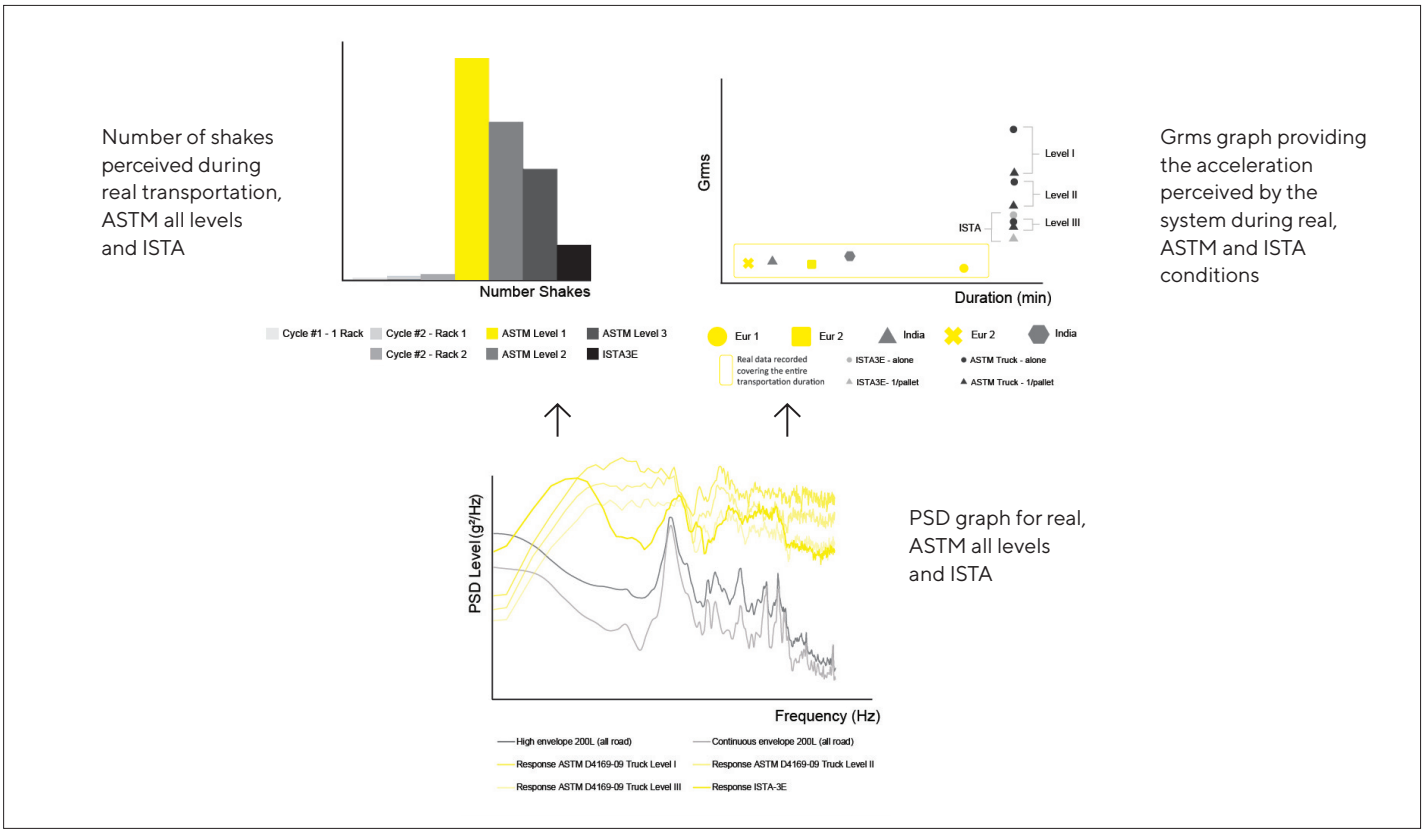


Figure 10: Flexsafe® 200L shipping system – data analysis example for Flexsafe® 200L system

This comparison allows the selection of the right parameters and configuration for worst case conditions of the qualification tests. The impact of real transportation is considerably lower than the impact of the ASTM all levels or ISTA norms. Comparison has been performed as well for the horizontal and vertical shocks (examples shown below):

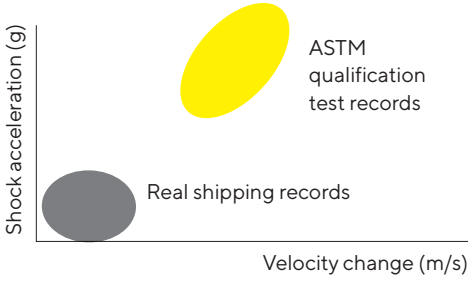


Figure 11: Horizontal shocks comparison between real trasnportation and ASTM D4169-14 all levels and ISTA 3E

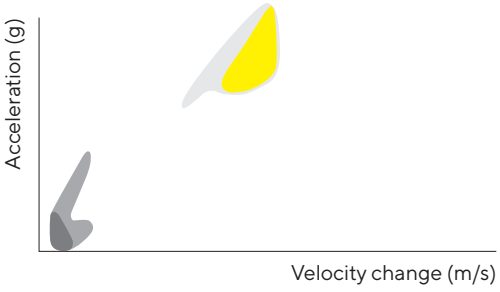


Figure 12: Vertical shocks comparison between real trasnportation and ASTM D4169-14 all levels and ISTA 3E

3.2 Truck vibratory profile

In this section, focus is made on vibrations generated by truck phases. The aim is to compare real measurements to laboratory measurement performed according to ASTM D4169-14 and ISTA 3E.

Both representations are an equivalent way to characterize the time signal in the frequency mode. Both PSD are compared to the ones measured according to ASTM D4169-14 (all levels) and ISTA3E.

3.2.1 Power Spectral Density (PSD)

Two PSD curves per configuration corresponding to real measurements are represented in the following graph:

- The light grey one is the PSD measured according to "continue" sampling (30s of record within 2 or 5 minutes).
- The dark grey one is the PSD measured according to "high" sampling (record when acceleration > 0.3 g).

Cycle 1: Real measurements versus laboratory measurement performed without pallet

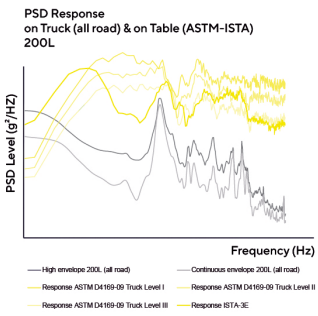
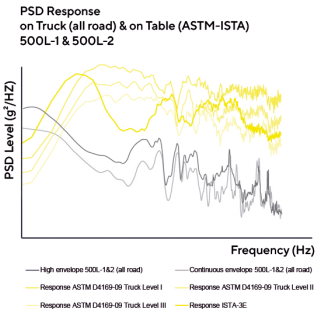
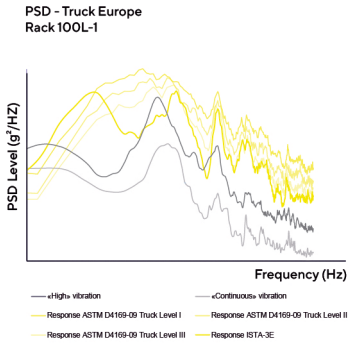
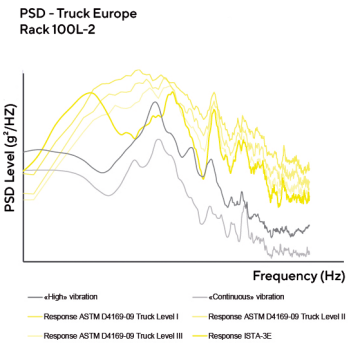
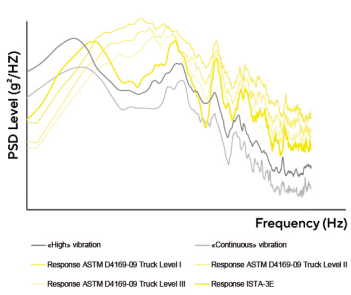
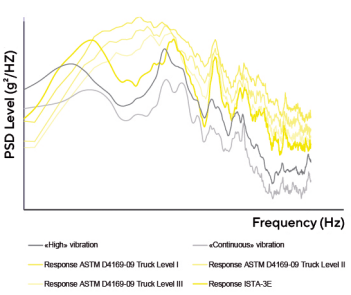
200L		500L 

Table 9: Cycle 1 analysis

The analysis below is performed according to this two sub cycles: Europe and India.

Cycle 2: Real measurements versus laboratory measurement performed with 1 system per pallet

Vol.	Cycle	Rack #1	Rack #2
100L	Europe		
	India		

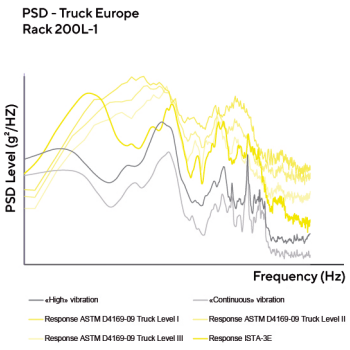
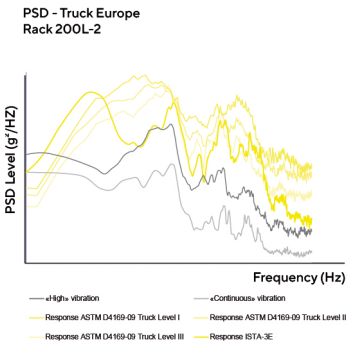
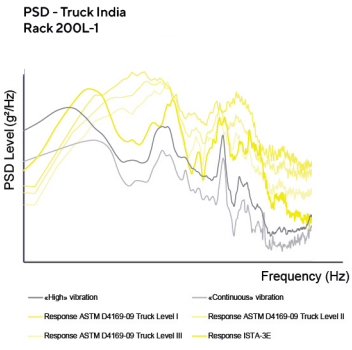
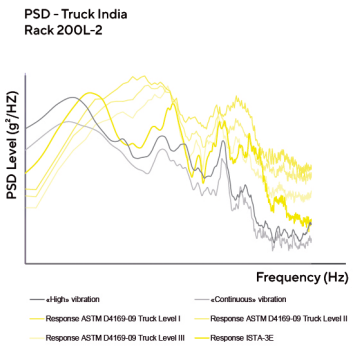
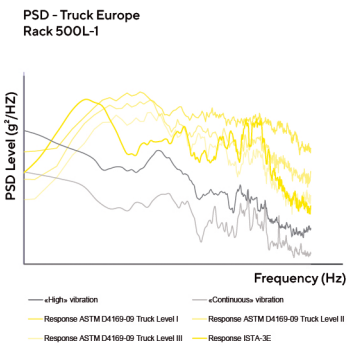
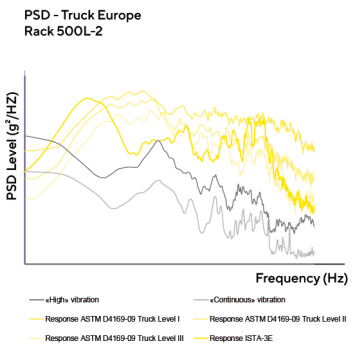
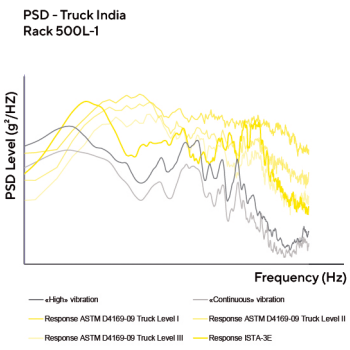
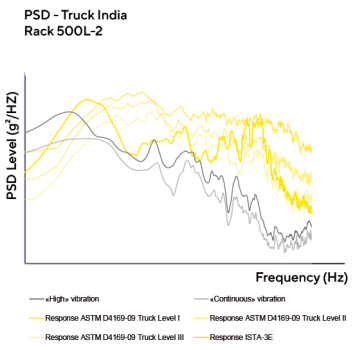
Vol.	Cycle	Rack #1	Rack #2
200L	Europe		
	India		
500L	Europe		
	India		

Table 10: Cycle 2 analysis

The real records are far below the records when the system is tested according to ASTM or ISTA norms, except for low frequencies where the shakes are the important factor. In conclusion, ASTM D4169-14 level III is (volumes and configurations) a appropriate level to be used for qualification. Level I and II provides even higher buffer margin.

3.2.2. Grms and duration

In this section, the RMS value of the PSD measured during real transportation is compared to the one seen during the laboratory testing according to the duration of application, since the PSD graph cannot provide this information.

After data analysis with experts, the Grms "high" record from real shipment is the more relevant comparison point with norms. Real shipping results and ASTM D4169-14 applied during 90 min are compared in the following graphs. This comparison allows to confirm the validity of this duration that has been used for the final qualification protocol.

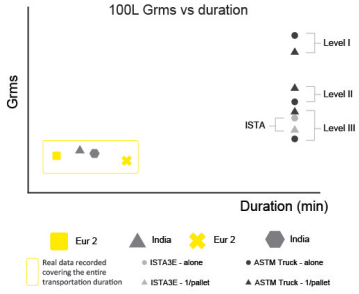
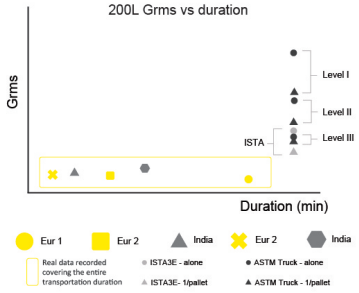
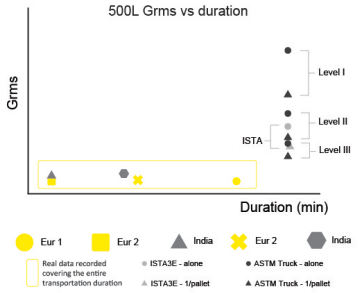
Flexsafe® System	Acceleration (Grms) graph comparing real conditions and ASTM D4169 or ISTA 3E truck solicitations (for comparable duration: 90 min)	
Flexsafe® 100L		90 min testing cover with very high buffer margin, the impact seen by the load during the real transportation tests in duration and in acceleration intensities.
Flexsafe® 200L		90 min testing cover with normal to very high buffer margin, the impact seen by the load during the real transportation tests in duration and in acceleration intensities.
Flexsafe® 500L		90 min testing cover with normal to very very high impact level, the impact seen by the load during the real transportation tests in duration and in acceleration intensities.

Table 11: Grms analysis between real transportation and testing according to ASTM D4169-14 all levels and ISTA 3E

In conclusion, the averaged intensity (RMS) of real vibration solicitations is always below the one generated during ASTM D4169-14 and ISTA 3E. Applying 90 min of ASTM D4169-14 truck profile at level II, guaranties very high impact leve versus real transport conditions, especially in terms of severity (high Grms). The choice of ASTM D4169 for truck profile without pallet provides more severe conditions than ISTA 3E testing.

3.2.3. Shakes

In addition of PSD and Grms, number of shakes is an indicator of vibration severity, particularly in low frequencies range. In the following graph, the number of shakes per cycles and volumes is compared to the number of shakes generated in 90 minutes of ASTM D4169-14 and ISTA 3E testing.

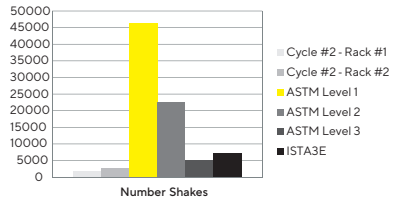
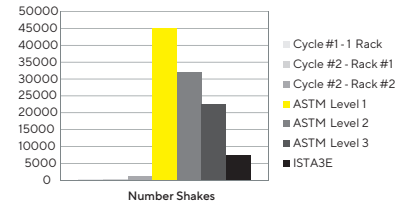
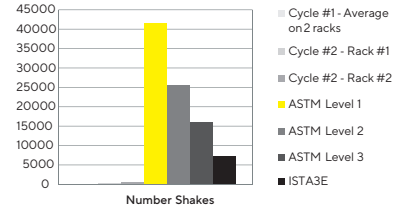
Flexsafe® System	Shakes graph comparing real conditions and ASTM D4169 or ISTA 3E solicitations (for comparable duration: 90 min)	
Flexsafe® 100L	100L – Truck – Number of shakes ASTM D4169 | ISTA 3E (no pallet) vs real 	The number of shakes during real transport conditions (< 4,000) are far below the number of shakes of the system during 90 min of ASTM D4169 level II (90 min) for truck at 22,500 = 6 times higher than the worst real conditions. ISTA 3E shakes (90 min) generates 2 times the worst level of shakes during real testing
Flexsafe® 200L	200L – Truck – Number of shakes ASTM D4169 | ISTA 3E (no pallet) vs real 	The number of shakes during real transport conditions (<1,800) are far below the number of shakes of the system during 90 min of ASTM D4169 level II for truck at 32,000 = 18 times higher than the worst real conditions. ISTA 3E shakes (90 min) generates 8 times the worst level of shakes during real testing
Flexsafe® 500L	500L – Truck – Number of shakes ASTM D4169 | ISTA 3E (no pallet) vs real 	The number of shakes during real transport conditions (<1,100) are far below the number of shakes of the system during 90 min of ASTM D4169 level II for truck at 25,650 = 23 times higher than the worst real conditions. ISTA 3E shakes (90 min) generates 9 times the worst level of shakes during real testing

Table 12: Shakes analysis between real transportation and testing according to ASTM D4169-14 all levels and ISTA 3E

Conclusion

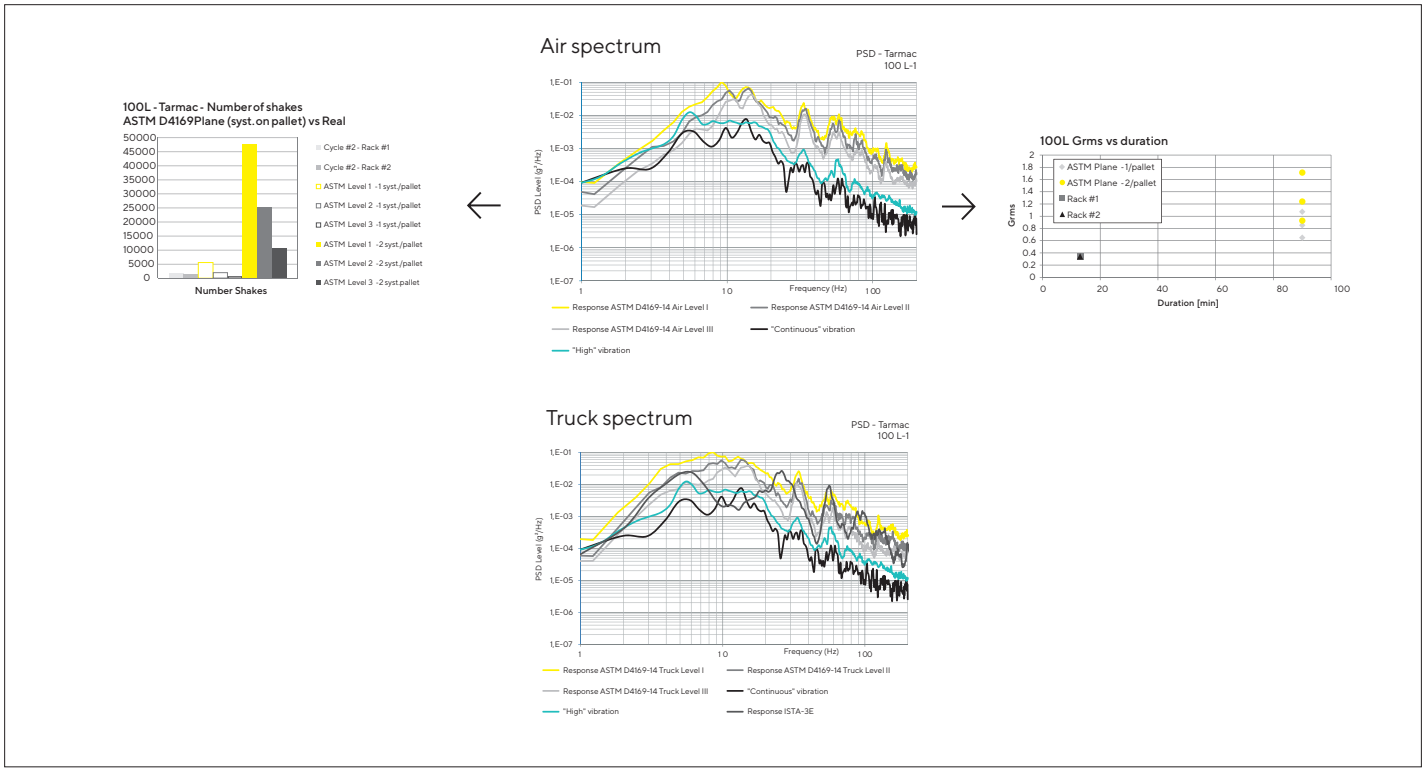
In the worst case configuration (without pallet), the number of shakes perceived by the Flexsafe® system under ASTM D4169 all levels or ISTA 3E is considerably higher than the real transport conditions.

3.3 Tarmac phase vibratory profile

In this section, focus is made on vibrations generated during tarmac phases. These phases have been analyzed due to the high amount of solicitation received by the loads and the worst case determination needed to be adapted to this type of vibrations. During the transportation of the systems on the tarmac type of road in the airports, the vibrations provided data for vibratory profiles (PSD), average intensity (Grms) and number | intensity of shakes.

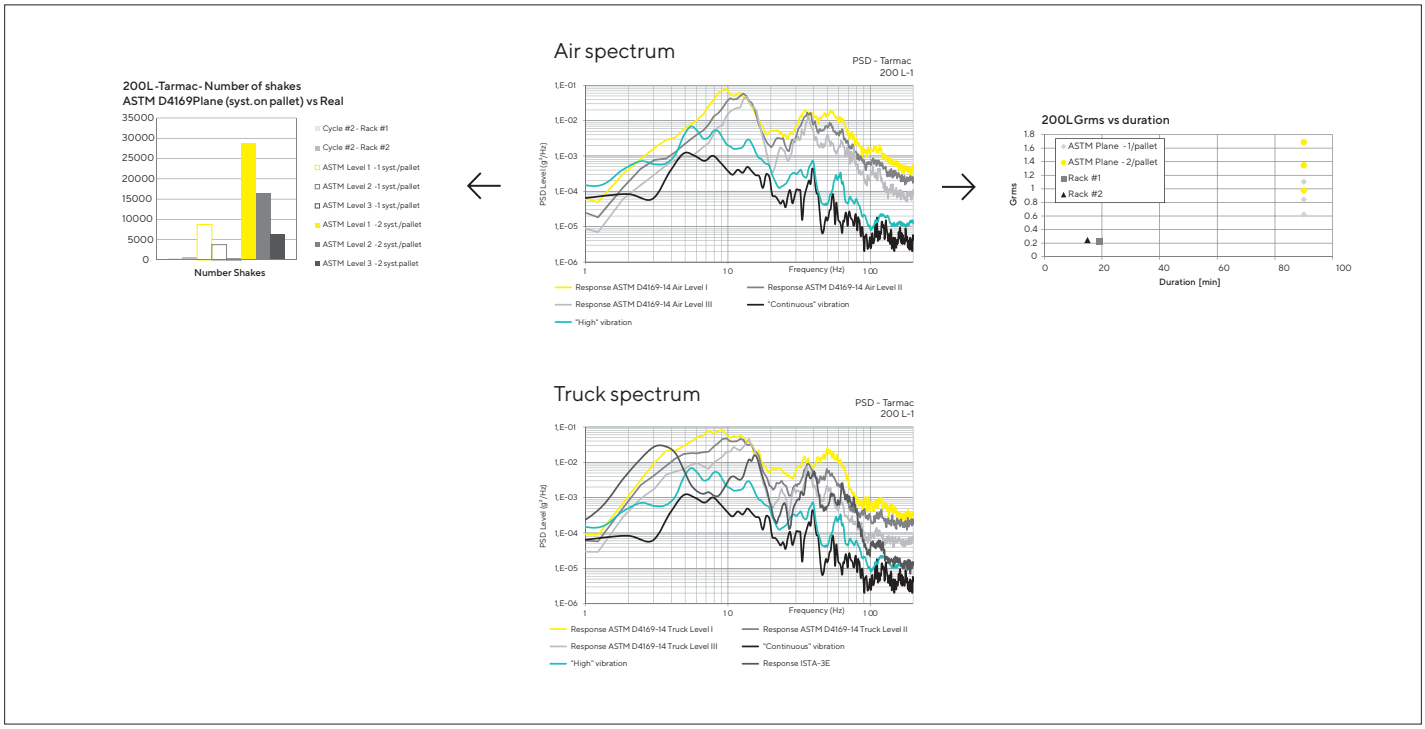
3.3.1 Results

100 L system



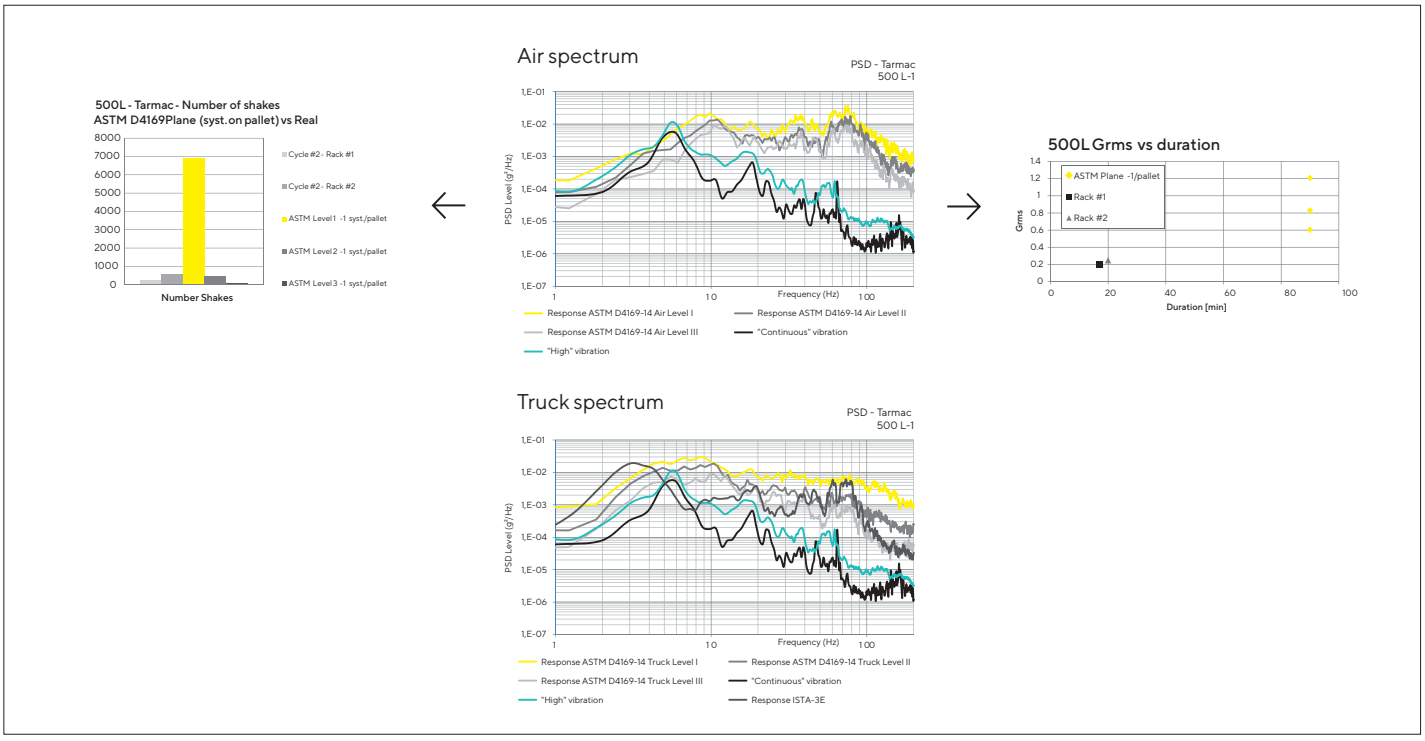
The number of shakes and Grms are significantly higher for 2 systems per pallet, thus defining the worst case configuration for 100 L Flexsafe® bags qualification when transported by airplane.

200 L system



The number of shakes and Grms are significantly higher for 2 systems per pallet, thus defining the worst case configuration for 200 L Flexsafe® bags qualification when transported by airplane.

500 L system:



3.3.2 Worst case determination for tarmac phase

For the tarmac, the worst case configuration is the conditions where we have the highest number of systems per pallet (2 systems per pallet for 100L and 200L, one system per pallet for 500L). The vibratory profile, the Grms and the number of shakes perceived by the Flexsafe® system under ASTM D4169 level II is higher than the real transport conditions, especially for the tarmac phase.

Conclusion:

The worst case conditions for the 100L and 200L systems is “without pallet” for the truck profile and with “two containers per pallet” for the air profile. The worst case conditions for the 500L system is “without pallet” for the truck profile and with “one container per pallet” for the airplane profile.

ISTA 3E is less severe than ASTM D4169 level II irrespective of the test condition for all Palletank configurations

- For the vibratory profile (PSD),
- For the Grms,
- For the shakes intensities and quantity. ISTA 3E is below or equivalent to what can be seen in level III.

According to these data, ASTM D4169 level II is more severe than ISTA3E in term of vibratory profile and horizontal or vertical shock solicitations.

Final condition choice for the future qualification testing program of the Flexsafe® shipping system:

The condition choice has been made, considering the following:

- According to best practice recommendations for transportation, all parcels should be installed in a pallet for any transport conditions (truck and plane),
- For truck condition, sometimes pallets are not used and the system is directly in contact with the roof of the truck,
- For plane transport, shipped system is always installed on a pallet,

In addition:

- Whatever the shipped conditions, testing of a shipped system without pallet is considered as a worst case condition,
- When the shipped system is installed on a pallet, when applicable (100L and 200L) testing of two shipped systems per pallet is worst case configuration compared to testing of one shipped system per pallet,

Finally, the qualification of shipped system has been chosen taking into account the worst case and the usual shipping condiotins. The tests have been carried out according to ASTM D4169- 2014 level II:

- Without pallet for handling protocol
- Without pallet for vibratory protocol according to truck conditions
- With a maximum of container per pallet for vibratory protocol according to plane conditions (2 systems per pallet for 100L & 200L and 1 system per pallet for 500L).

4. Qualification of Flexsafe® 3D Bags 100L to 500L in Shipping Palletank

This analysis based on the comparison between measurements on real transportation and laboratory transport simulation in addition of ASTM recommendations for validation of transportation was made to support the definition of qualification protocol of shipping systems.

4.1 Qualification protocol description

4. System qualification

Filled Flexsafe® 3D bag qualification according to ASTM D4169-2014 assurance level II for cycle 12 & 14 at 4°C (39.2°F) and 40°C (104°F).

Test sequence		Test reference based on ASTM D4169-14 – Assurance level II
1	Pre-conditioning	4°C (39.2°F) or 40°C (104°F) during 72 hours
2	Mechanical handling	SCHEDULE A
2.1	Truck handling	ASTM D6055 Method A | 5 cycles round trip
2.2	Horizontal impact	ASTM D880 Method B | each side 1 shock (total 4)
2.3	Rotational flat drop test	ASTM D6179 Method C | 2 drop tests (short and long edge)
3	Vehicle vibration*	SCHEDULE E
3.1	Truck spectrum	ASTM D4728 Method A – 90 minutes truck spectrum
3.2	Air spectrum	ASTM D4728 Method A – 90 minutes airplane spectrum
4	Low pressure	SCHEDULE I: ASTM D6653 40°C and 4°C – 595.73hPa – 60 minutes
5	Mechanical handling	SCHEDULE A
5.1	Truck handling	ASTM D6055 Method A | 5 cycles round trip
5.2	Horizontal impact	ASTM D880 Method B | each side 1 shock (total 4)
5.3	Rotational flat drop test	ASTM D6179 Method C | 2 drop tests (short and long edge)
6	Warehouse stacking	SCHEDULE B: ASTM D642 – during 3s

Table 13: Test sequence based on ASTm D4169-14 cylce 12 & 14

* Note that rail transportation is not considered in shipping validation.
Truck and Air testing are higher than rail transportation solicitation and largely cover this type of shipment.

4.2 Qualification protocol rational

4.2.1. Testing sequences definition

The validation protocol is based on ASTM D4169-14. Distribution Cycles #12 and #14 have been selected as the closes to the usual transportation conditions for the Biotech industry:

#	Distribution cycle
12	Air (intercity) and motor freight (local), over 150 lb. (68.1 kg), or unitized
14	Warehousing (partial cycle to be added to other cycles as needed)

Table 14: Choice of ASTM D4169-14 distribution cycles

The testing sequences of Distribution Cycles #12 and #14 have been displayed on the usual transportation condition:

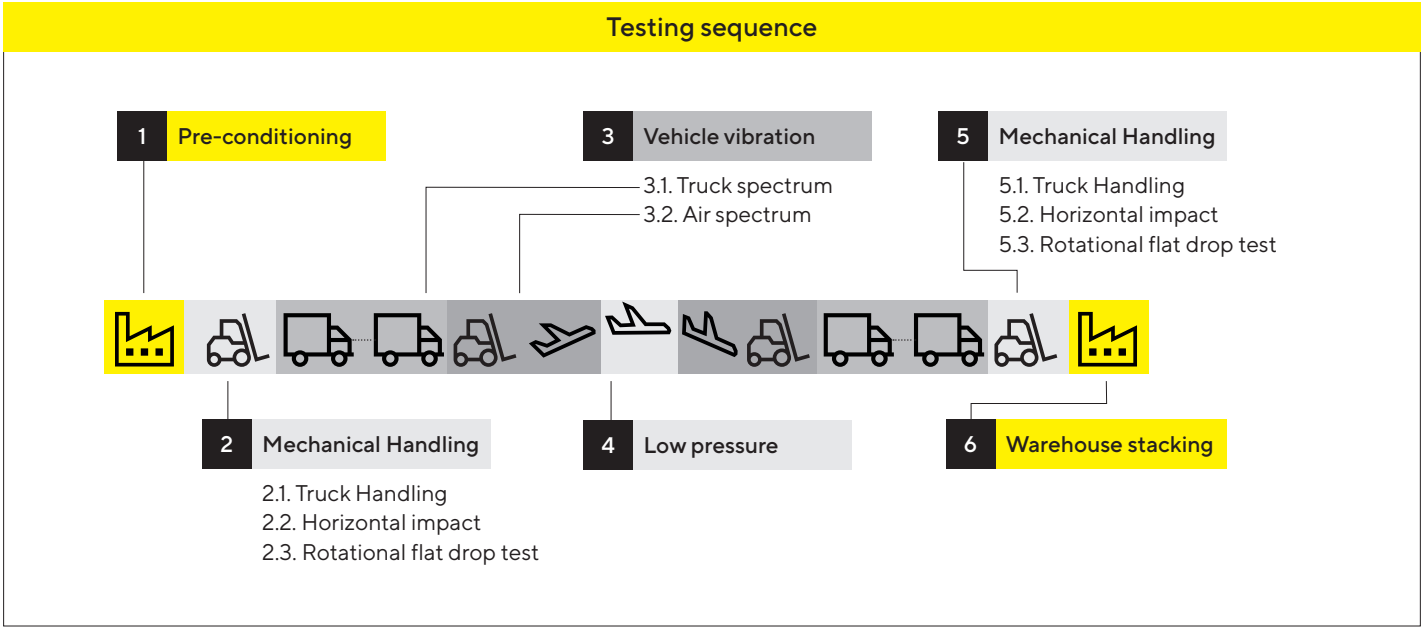


Figure 13: Test sequence linked to the usual transportation means in Biotech

The combination of cycles 12 and 14 provide the testing sequence for the final qualification of the Flexsafe shipping systems (table 13)

4.2.2 Testing samples and conditions

With the knowledge acquired during the preliminary testing, the worst case conditions have been chosen for the qualification testing program:

- Flexsafe® filled 100L, 200L and 500L bags in their shipping Palletank without a pallet for truck vibration spectrum
- Flexsafe® filled 100L, 200L bags in their shipping Palletank with two systems per pallet for air vibration spectrum
- Flexsafe® filled 500L bags in their shipping Palletank with one system per pallet for truck vibration spectrum.

Flexsafe® 3D bags are gamma sterilized under a worst case dose: 50kGy. 4 bags per volume and per temperature (4°C and 40°C) are tested. The same bags are tested after 1 year of accelerated ageing and after 3 years of accelerated ageing (in progress).

4.2.3 Acceptance criteria

With the knowledge acquired during the preliminary testing, ASTM D4169 cycle 12 and 14 with acceptance level II has been chosen as being the qualification level providing buffer margin.

Acceptance level I is highly severe and was achieved for all volumes in the preliminary testing (one system per volume). Acceptance level I was tested repetitively, but demonstrated a risk of structural damage to the Stainless Steel Palletank.

ASTM D4169-14 acceptance level II has been taken as the qualification level, which is the most severe testing program. ASTM D4169-14 Level III could have been used for qualification as well: PSD, Grms, Shakes and Shocks are higher than the real conditions. Choosing ASTM D4169-14 level II for Truck and Air provides even very high impact leve for the Flexsafe® liquid shipping system qualification.

After the ASTM tests cycles, the systems have been analyzed for the absence of damage and the bags have been inspected for the absence of leakage by visual inspection and dye penetration test (Shipping validation report N° SD144680).

Acceptation criteria after the tests:

- Visual inspection during test – no leak, no damage on the system
- Visual inspection of the emptied bag – no leak
- Dye penetration test on emptied bag – no leak

4.3 Qualification Test results ASTM D4169

- Shipping of liquid filled bags – ASTM D4169 cycle 12 & 14, level II after storage during 72 hours at 4°C (39.2°F):

Ageing	Test samples and number (50kGy) 100L | 200L | 500L	Container	Total	Result
T=0	4 bags per volume	Shipping	12	Compliant to specifications for systems & bags – no leak
T=1 year	4 bags per volume	Shipping	12	Compliant to specifications for systems & bags – no leak
T=3 years	4 bags per volume	Shipping	12	Compliant to specifications for systems & bags – no leak

Table 15: Qualification tests results at 4°C (39.2°F)

- Shipping of liquid filled bags – ASTM D4169 cycle 12 & 14, level II after storage during 72 hours at 40°C (104°F):

Ageing	Test samples and number (50kGy) 100L | 200L | 500L	Container	Total	Result
T=0	4 bags per volume	Shipping	12	Compliant to specifications for systems & bags – no leak
T=1 year	4 bags per volume	Shipping	12	Compliant to specifications for systems & bags – no leak
T=3 years	4 bags per volume	Shipping	12	Compliant to specifications for systems & bags – no leak

Table 16: Qualification tests results at 40°C (104°F)

Flexsafe® 3D bags in the shipping Palletank provide reliable liquid shipping qualified according to ASTM D4169 cycles 12 & 14 at the assurance level II for a temperature range of 4°C (39.2°F) to 40°C (104°F).

Conclusion

1. Real shipping conditions
Analysis of acceleration measured on filled Flexsafe® shipping system during real transportation (including handling, truck and airplaine shipments).

2. Laboratory tests with norms
Analysis of acceleration measured on filled Flexsafe® shipping system during testing in laboratory according to ASTM D4169-2014 at different severity levels and ISTA 3E.

3. Qualification protocol determination
Real transport cycle analysis and comparison with laboratory testing results in order to choose the right setting parameters for Flexsafe® bags qualification with ASTM D4169-2014.

4. System qualification
Filled Flexsafe® 3D bag qualification according to ASTM D4169-2014 assurance level II for cycle 12 & 14 at 4°C (39.2°F) and 40°C (104°F).

The selection of proven and robust single-use solutions provides end-users with reliable and easy-to-use handling systems to minimize risk of product loss. Long-term product integrity and stability is maintained and contained international shipments are ensured.

Flexsafe® 3D bags in the Sartorius Stedim Biotech shipping Palletank are qualified for liquid shipping at nominal volume under the international norm ASTM D4169-14 level II for cycle 12 & 14 at 4°C (39.2°F) and at 40°C (104°F) and provide contained liquid shipping for all biotech fluids.

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