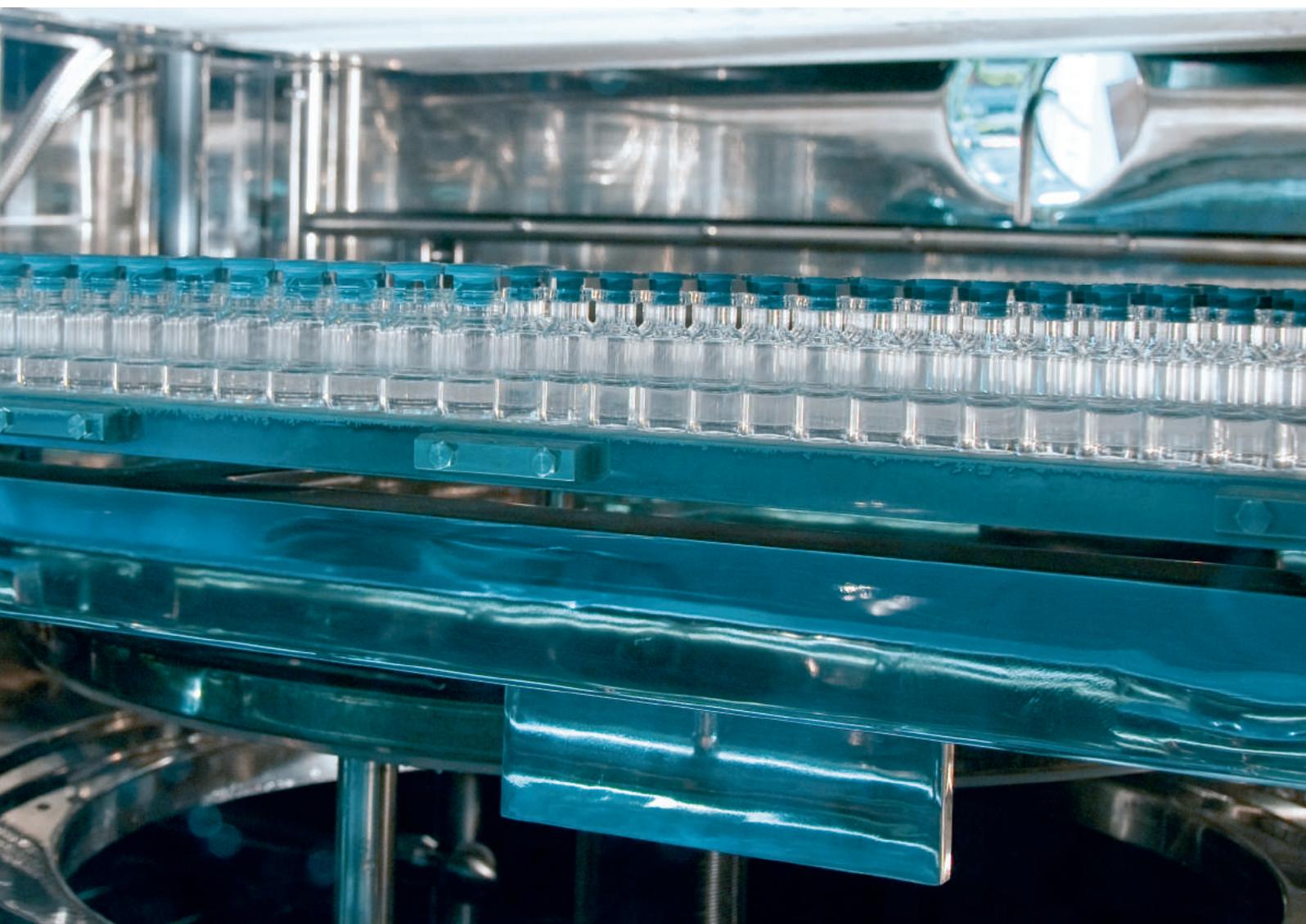


VERISEQ[®] Nucleation.

Improved lyophilisation through
controlled nucleation.



Importance of controlled nucleation.

Improving results with VERISEQ® Nucleation

Lyophilisation, or freeze drying, is an important downstream process for stabilising pharmaceutical compounds.

The control and repeatability of lyophilisation cycles are crucial for consistently achieving high product quality. Although the primary and secondary drying steps may be well controlled, the lack of control of the ice nucleation temperature (the temperature at which the freezing starts) can adversely affect product uniformity and lead to suboptimal freeze drying cycles. Colder nucleation temperatures cause ice crystals to be smaller, thus leading to a higher resistance to vapour flow, longer cycles, possible vial breakage as well as a larger vial-to-vial variability.

Demand for uniformity is growing because of the high value of drugs being lyophilised. Also, regulatory bodies are putting pressure on manufacturers to better control and understand their processes.

VERISEQ Nucleation is an advanced innovative controlled nucleation technology capable of producing sterile cryogenic ice fog and circulating it within a lyophiliser chamber to ensure reliable and prompt top-down nucleation of the vial-contained pharmaceutical formulations at an optimal temperature. As a result, overall cycle time is reduced by up to 35%, vial-to-vial variance is reduced, product uniformity is improved, ice crystal size is increased, leading to reduced water vapour resistance, and reconstitution time is also decreased. The technology is applicable to laboratory-, pilot- and production-scale lyophilisers.

Nucleation is a crucial step in the freeze drying process



Step 1: initial product



Step 2: freezing
(nucleation & solidification)



Step 3: primary drying



Step 4: secondary drying



Step 5: final product

Nucleation basics.

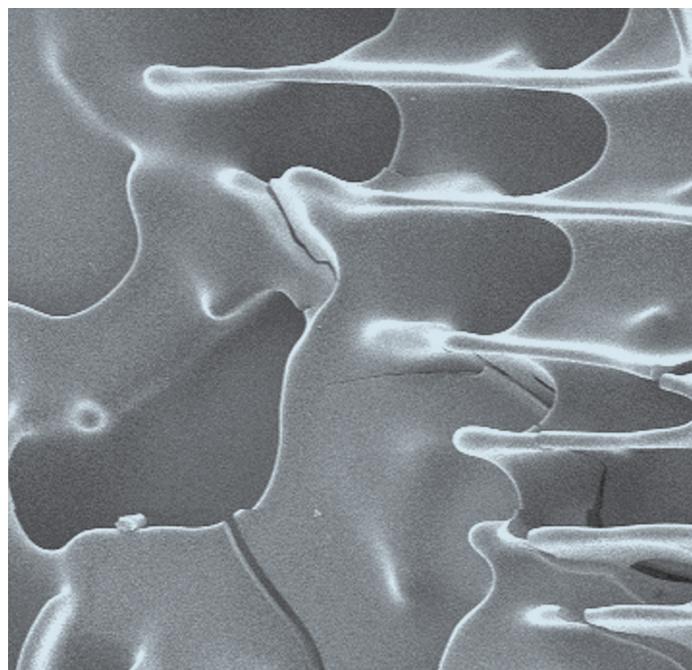
Nucleation is the process by which ice crystals (seed crystals) are formed from originating sites called nuclei.

In nature, suspended particles or container defects provide nucleation sites.

In pure systems (like sterile liquid pharmaceuticals), stable nuclei form from frozen solvent, but only after significant supercooling. Supercooling impacts the size of ice crystals, and solutions that nucleate at different supercooled temperatures will have different ice crystal sizes.

When allowed to nucleate naturally, each container in a batch of product will nucleate randomly at a different temperature. Subsequently, these vials dry at different rates over an extended period of time and therefore their uniformity varies (see also graphics on page 5).

VERISEQ Nucleation has been developed by Linde to control the nucleation process and therefore improve the lyophilisation process and the end product.



Magnified ice crystal after controlled nucleation with VERISEQ® Nucleation: Improved pore structure resulting in larger pores, smaller surface area and lower water vapour resistance.

Freezing step using VERISEQ® Nucleation



Prior to ice fog injection/product in liquid state



During ice fog injection/ice nucleation in progress



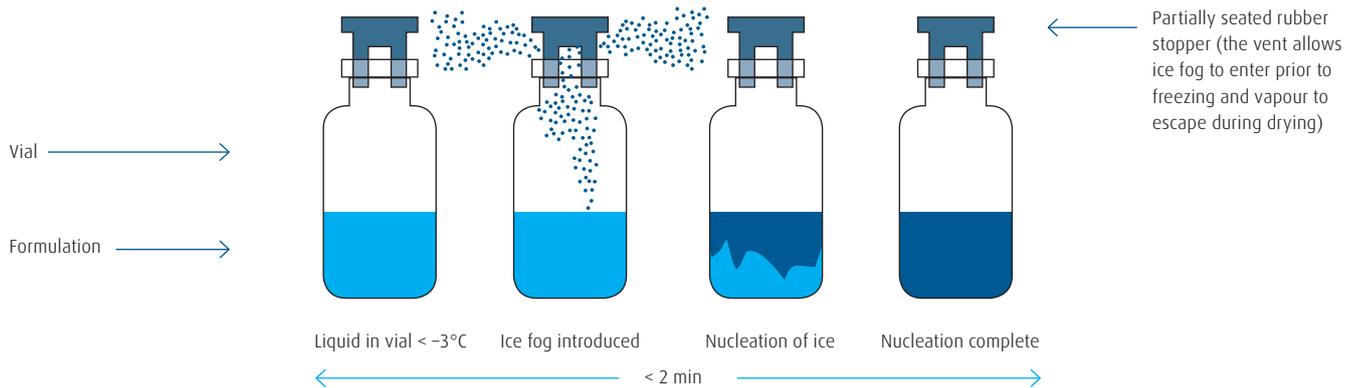
After ice fog injection/all vials nucleated

VERISEQ[®] Nucleation process.

Nucleation process

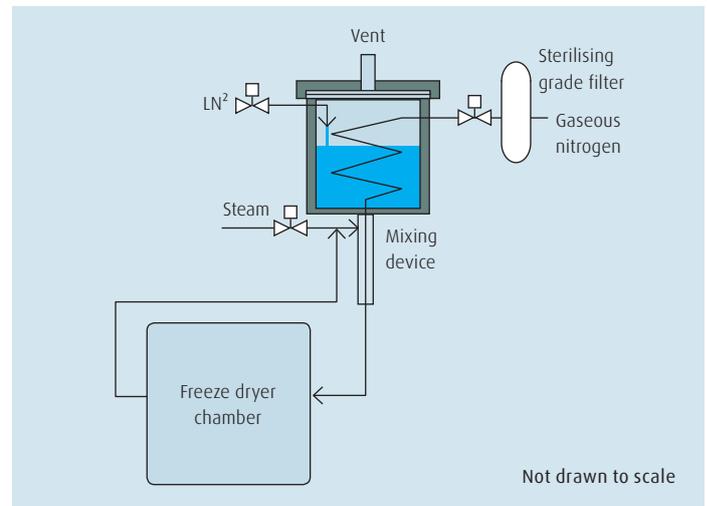
VERISEQ Nucleation uses liquid nitrogen and water vapour to create a cryogenic fog or "ice fog". This fog permeates the product chamber and enters the vials containing the supercooled liquid solution.

As soon as the ice crystals contact the surface of the product, nucleation proceeds rapidly from the top of the vial to the bottom. The performance is independent of the vial type (tubing vs. moulded).



Aseptic system

The production-scale VERISEQ Nucleation system is a sterilisable system. The liquid nitrogen is produced by liquefying gaseous nitrogen which has been sterilised by filtration. Rather than using water vapour to create the cryogenic fog, the aseptic system utilises clean steam that is normally used to steam-sterilise the freeze dryer. This system can be fully integrated into a new freeze dryer or added to an existing one.



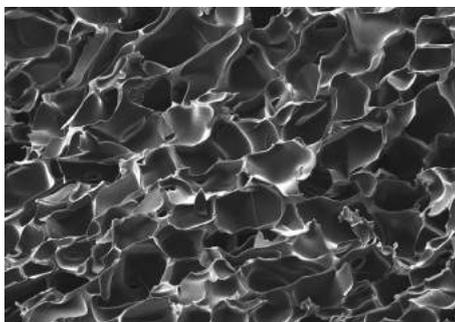
Summary of lab-scale testing

Product Types	Tests	Results	Freeze Dryer Shelf Area
Mannitol	Identical cycles with and without VERISEQ	Improved drying profile as evidenced by TDLAS and product probes	0.1 m ² , 2.3 m ²
Sucrose	Identical cycles with and without VERISEQ	Improved drying profile as evidenced by TDLAS and product probes	2.3 m ²
Mannitol	Full-scale test on installed 28 m ² freeze dryer	100% nucleation in 15,000 30 ml vials	28 m ²
Antibiotic (tricyclic)	Comparison cycles followed by an optimised cycle	33% reduction in cycle time	2.3 m ²
Vaccine	Identical cycles with and without VERISEQ, scale up to 56 m ² machine	Improved drying profile, smaller variation in activity assay	2.3 m ² , 56 m ²
Amino acid	Identical cycles with and without VERISEQ	Improved drying profile as evidenced by TDLAS and product probes	2.3 m ²
Human serum albumin	Compared tubing and moulded vials with and without VERISEQ. Evaluated particulate impact	Reduced cycle time for moulded vials. No increase in particulate	2.3 m ²
Bacterial lysate	Bulk product evaluated for enhanced freezing profile	Frozen structure visibility improved	2.3 m ²
Tripeptide	Identical annealing cycles with and without VERISEQ	No reduction in cycle time	0.1 m ²
Monoclonal antibody	Comparison cycles followed by an optimised cycle	50% reduction in cycle time	2.3 m ²

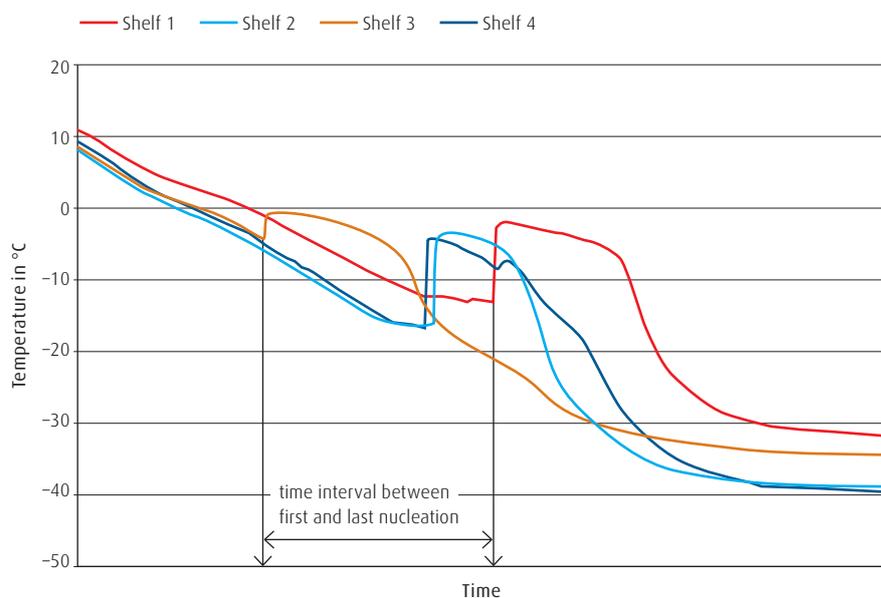
Advantages of VERISEQ[®] Nucleation.

Natural (spontaneous) nucleation

- Nucleation occurs randomly
- High vial-to-vial variability
- Significant supercooling = smaller ice crystals (see picture below)
- Smaller ice crystals = higher water vapour resistance
- High resistance = longer cycles = higher costs



Natural nucleation: smaller ice crystals, larger vial-to-vial variability. Same magnification as below.

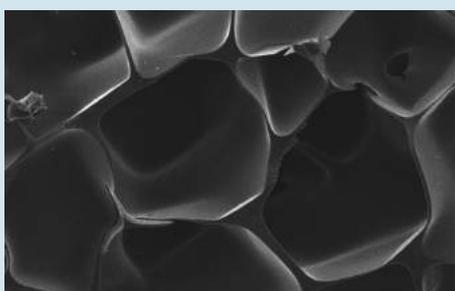


Product temperature profile across trays during freezing step. Actual data from customer test. Colours refer to temperature measurements of the reference vials. Thermocouples mounted on exterior of vials.

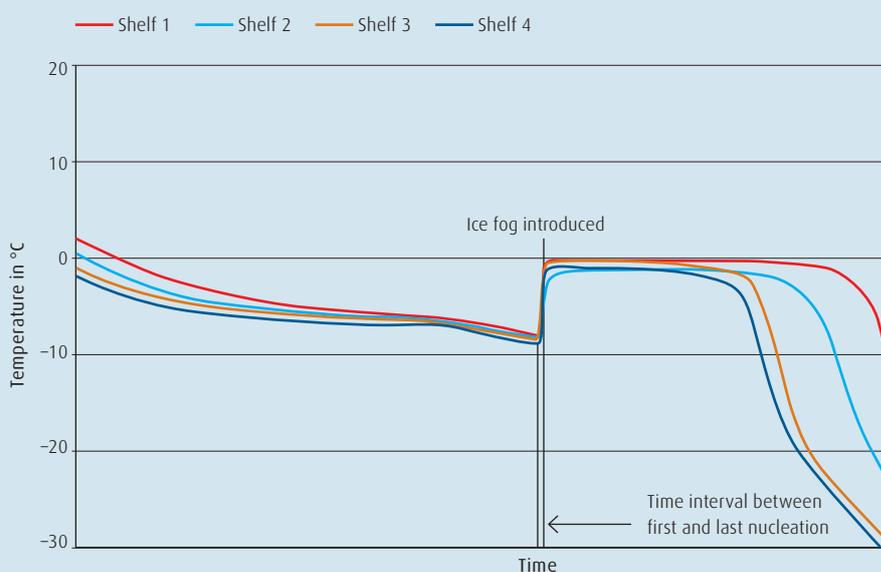
Controlled nucleation with VERISEQ Nucleation

With Linde's VERISEQ Nucleation technology, you can reduce your cycle time by up to 35% at improved quality levels.

- Reduced vial-to-vial variance leading to increased batch uniformity
- Shortened primary drying time and reduced overall cycle time (in some studies by 30–35%)
- Reduced reconstitution time
- Reduced scale-up inaccuracies
- Improved cake appearance
- Reduced water vapour resistance due to increased ice crystal size (see picture below)



VERISEQ[®] Nucleation: larger ice crystals, reduced vial-to-vial variance. Same magnification as above.



Product temperature profile across trays during freezing step. Actual data from customer test. Colours refer to temperature measurements of the reference vials. Thermocouples mounted on exterior of vials.

VERISEQ[®] Nucleation portfolio.

Thanks to our partnership with IMA Life, VERISEQ Nucleation can be implemented on virtually any freeze dryer. Our flexible solution can be installed in new units, retrofitted on existing systems and connected to multiple lyophilisers.

Sizing

- The VERISEQ Nucleation system is flexible and easily fits any size or configuration of freeze dryer
- It has been tested on freeze dryers ranging from 3 m² to 56 m²

Layout

- Requires limited space
- Complies with a variety of configurations

Versatility

- Easily retrofittable, using existing connections on a freeze dryer chamber
- Can accommodate varying filter rack layouts and utility supplies with local controls or an integrated system

VERISEQ Nucleation production-scale system

- Compact, integrated system
- Aseptic system
- Fully automated, recipe-selectable cycle
- All operations are conducted at or below atmospheric pressure
- Requires only two 50 mm (2") connections to chamber on chamber (larger connections preferred)
- Design allows steam in place (SIP) along with the freeze dryer chamber

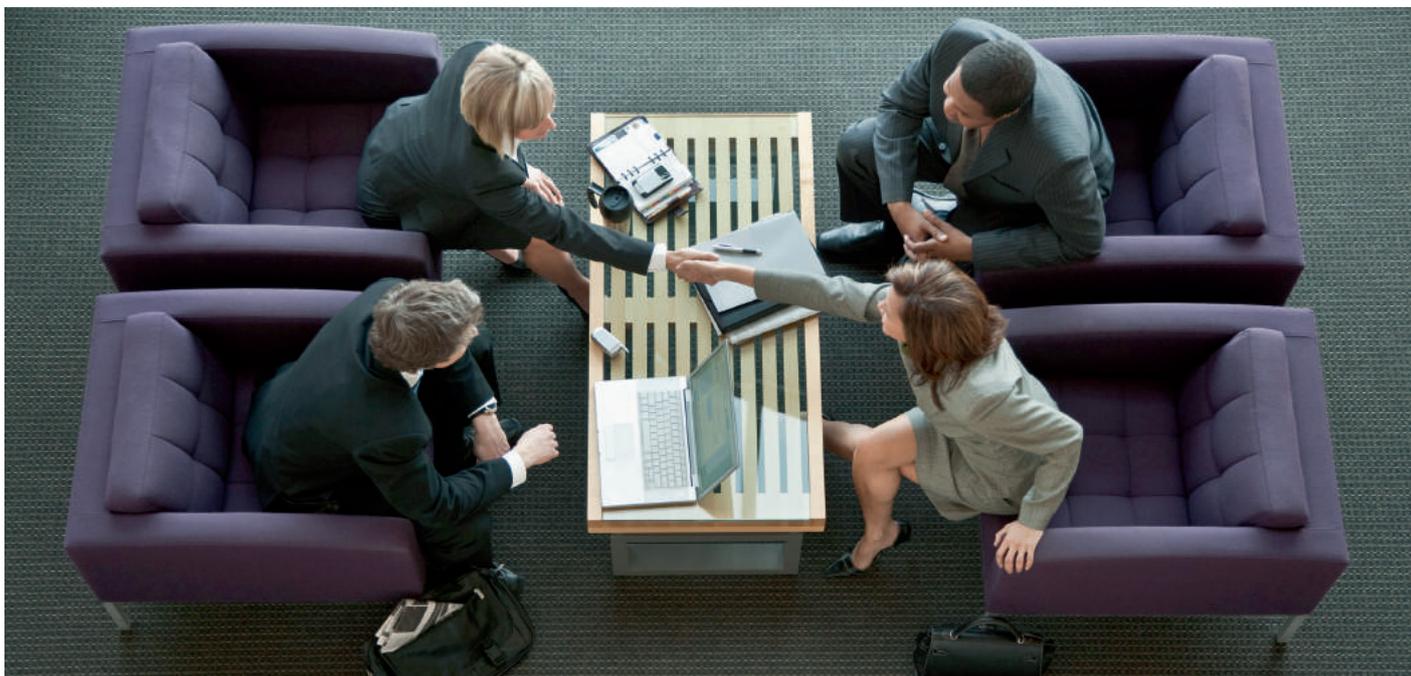


VERISEQ Nucleation for laboratories

- Compact, portable system
- Non-aseptic system
- All operations are conducted at or below atmospheric pressure
- Suitable for laboratories and pilot facilities, enabling them to test new formulations with controlled nucleation in the lyophilisation process
- Adaptable to all lab-/pilot-scale lyophilisation units; requires 2 x 1-1/2" ports
- Self-contained, fully automated one-button operation
- PLC-controlled with HMI interface
- Data logging



A strong partnership.



VERISEQ® Nucleation has been developed by Linde. The technology is exclusively distributed by IMA Life.

About Linde

Linde is a world-leading supplier of industrial, process and specialty gases and is one of the most profitable engineering companies. Linde has been supporting the pharmaceutical industry with pharmaceutical-grade gases for many decades and has gained in-depth understanding of the challenges pharmaceutical companies are facing.

- Linde's offering for the pharmaceutical industry includes:
- VERISEQ® process-grade gases – high-purity, traceable nitrogen, oxygen, carbon dioxide, argon, helium and synthetic air
- HiQ – specialty gases for calibrating gas monitoring equipment
- Process chemicals for applications like amination, catalysis or fluorination
- Gas applications for ozonolysis, inerting, purging, charging, mixing
- Gas applications for reactor cooling, cryogenic storage and transport, freezing
- Gas applications for freeze drying – cryogenic cooling and controlled nucleation in cooperation with IMA Life

About IMA Life

- Exclusive rights to market VERISEQ Nucleation technology
- World leader in the design and manufacture of aseptic processing and freeze drying equipment
- Five manufacturing sites in Italy, the US and China, and three service centres in Italy, the Netherlands and Japan
- Worldwide network covering more than 80 countries
- Access to freeze drying laboratory for development work with one laboratory-scale and one pilot-scale freeze dryer and other analytical tools including tunable diode laser absorption spectroscopy (TDLAS) instrumentation, residual gas analyser and other re-configurable instrumentation ports





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