PHYSICAL AND BIOLOGICAL COLLECTION EFFICIENCIES

TSI AEROTRAK+ REMOTE ACTIVE AIR SAMPLER MODEL 7010

APPLICATION NOTE CC-127
(5/6/2021) Rev B (A4)

Contents

Introduction—Microbial Air Sampling for Pharma

As part of good manufacturing practice (GMP) compliance, medical and pharmaceutical manufacturing operations must qualify and routinely monitor microbial contamination levels in cleanrooms and clean spaces such as isolators and restricted access barrier systems (RABS). The use of active air samplers (AASs) is an essential part of this process.

Even though impaction-based microbial samplers have been in use since the middle of the 20th century, currently there is no standard defining minimum performance requirements. As a result, there are significant differences in performance for AASs in use today, which can lead to improper evaluation of cleanroom microbial contamination levels. Therefore, proper characterization of microbial air sampler performance is a critical aspect of a contamination control program.

While no standards currently define minimum performance levels for AASs, ISO 14698-1[1] and EN 17141:2020[2] (which is scheduled to replace ISO 14698-1) provide recognized guidance regarding active air sampling equipment and validation. These standards describe two ways to evaluate the collection efficiency of microbial air samplers: physical efficiency and biological efficiency.

Physical efficiency defines how well the sampler collects different sizes of particles, regardless of the composition of the particles (inanimate, microorganisms, or microbe-bearing). The biological efficiency defines how well the sampler collects viable microbe-bearing particles which can form colony-forming unit (CFU). It includes the losses caused by both the physical efficiency and the effect that the sampling has on the viability of the microorganisms due to stressing during collection and dehydration of the media.

While physical efficiency can be measured using biological particles, often it is measured using polystyrene latex spheres (PSL) or other non-viable particles. Biological efficiency is measured using microbes. Annex E of EN 17141 provides a formula to calculate the d_{50} value, or physical efficiency, for AASs using impaction. In addition, Annex B of ISO 14698-1 and Annex E of EN 17141 define procedures that can be used to measure the physical and biological efficiency of a sampler.

TSI recently introduced a new AAS—the TSI AeroTrak®+ Remote Active Air Sampler (AAS) Model 7010. It uses external vacuum to draw air through the AAS. With an impaction velocity of 43 m/s, the AAS is designed to provide high physical and biological efficiency over a broad range of particle sizes. This document describes testing that was done to determine the performance of AeroTrak+ Remote AAS, and includes complete test results.

Background—Evaluating Air Samplers

The efficiency of a sampler can be defined as the number of particles captured in the sampler divided by the number of particles in the environment (for the same volume of air). Since sampling efficiency is normally tested at a variety of particle sizes, the results are typically plotted as sampler efficiency versus particle size.

The d_{50} value, also known as the impactor cut point, is used to describe the overall efficiency of a sampler. The d_{50} value is defined as the aerodynamic equivalent particle size at which 50% of the particles are collected and 50% pass by the culture medium. The lower the d_{50} value, the more efficient the sampler is at capturing particles. No acceptance criteria for the d_{50} value is explicitly stated in either standard; however, ISO 14698-1 suggests a sampler should collect particles down to 1 µm in size and EN 17141:2020 states a d_{50} value smaller than 2 µm is considered appropriate.

The impaction velocity is the velocity of the air (and particles) as they exit the nozzles (openings) on the sampler. Impact velocity is incorrectly defined in ISO 14698-1 as the velocity of the air hitting the culture medium. The air does not “hit” the culture medium. The air turns as it approaches the surface, flowing parallel to the surface. Particles, because of their inertia, are unable to make the turn and impact onto the culture medium. This is the principle that all impactors work on. The impaction velocity is the maximum velocity at which larger particles will hit the culture medium. As particle size decreases, they begin to follow the air as it turns, reducing the velocity at which the particles hit the culture medium. The higher the impaction velocity, the smaller the particle size that will be captured by the impactor. The impaction velocity is a compromise. Too high of a velocity can cause damage and affect the viability of the viable particles. Too low of a velocity and the viable particles will not be captured. The d_{50} value is affected by the impaction velocity and the geometry of the sampler, with a key parameter being the distance from the nozzle exit to the agar plate.

The biological efficiency of a sampler is a function of the physical efficiency of a sampler. Biological efficiency is lower than the physical efficiency due to damage of microorganisms during capture or inability of the collection medium to promote growth. Because physical efficiency is a function of particle size, the same can be said for biological efficiency. It is important to look at the size of the biological particles used in testing. Ideally the data is presented in a graphical format, showing efficiency versus particle size. If the data is presented as a single efficiency number, then the user must consider the particles sizes involved in the testing. A sampler with a biological efficiency of 90% tested with 5 µm particles may not be as efficient overall as a sampler with an efficiency of 80% that was tested with 1 µm particles.

A properly designed AAS has two important main characteristics: the impaction velocity and d_{50} value. In Figure 1, the relative recovery rate for *P. fluorescens* and *M. luteus* for various velocities are empirically determined by Stewart et al[3]. The relative recovery rate is calculated by the number of colonies on the collection medium relative to the number of bacteria entering the AAS. These tests, and other similar peer reviewed publications, consistently indicate that an impaction velocity between 30-50 m/s is desired for maximizing biological efficiency with a velocity near 40 m/s being ideal. Many AASs in the market have low impaction velocities (around 10-20 m/s) resulting in bacteria not impacting on collection media while others have high impaction velocities (greater than 50 m/s) causing injury to organisms leading to artificially low colony counts. Many manufacturers in the market do not publish a d_{50} value in their AAS literature due to the high d_{50} values these instruments have. Without this information, proper performance evaluation across different organism types cannot be claimed.

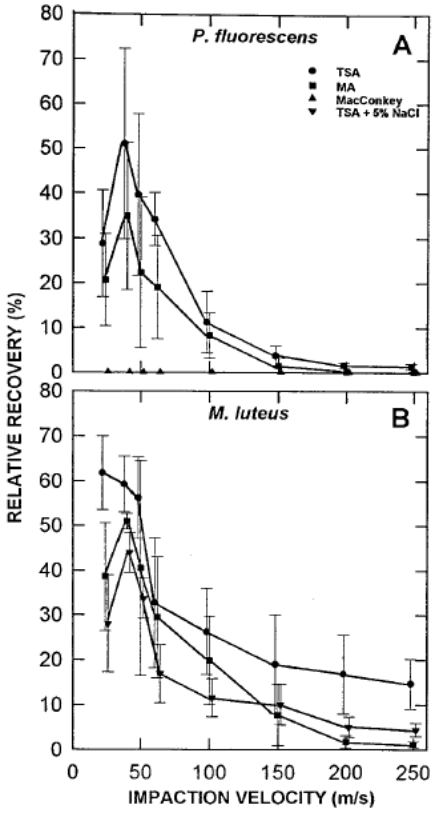

Figure 1: Relative recovery rate across various impaction velocities for P. fluorescens and M. luteus

Calculation of the d_{50} Value—Proof in Testing

EN 17141 standard gives a simplified formula to calculate an impaction AAS's *d_{50} value*:

$$d_{50} = \sqrt{\frac{40 \times Dh}{U}}$$

Where 40 is a constant from the air viscosity (°C)
Dh is the Hydraulic Diameter, or diameter of circular holes (mm)
U is the Impaction Velocity (m/s)

For the AeroTrak+ Remote AAS, the d_{50} is calculated as:

$$d_{50} = \sqrt{\frac{40 \times 0.855}{43}}$$

This formula calculates the d_{50} value to be 0.89 µm.

Test Procedures for the Experimental Determination of the d_{50} Value

The physical efficiency data for the AeroTrak+ Remote AAS was taken using three different experimental test methods. The first test method utilized oleic acid particles generated with a TSI Flow Focusing Monodisperse Aerosol Generator (FMAG) Model 1520. Testing was performed with particles sizes over the range of 0.7 µm to 1.5 µm. The output of the FMAG was sampled by the AAS. A filter was located downstream of the AAS to capture particles leaving the AAS. A schematic of the test set-up is shown in Figure 2.

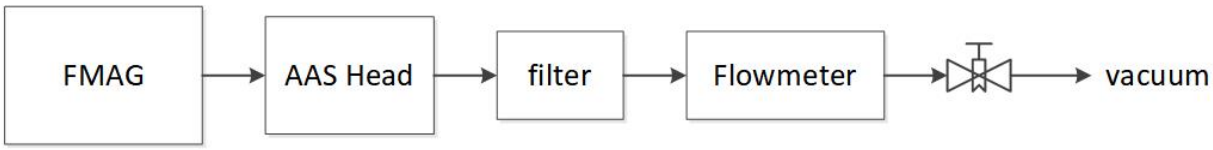

Figure 2: Test set-up for measuring physical efficiency with oleic acid particles.

The collection efficiency of the AAS head was determined by the fluorometric method as described by Chen et al[4]. For each particle size, monodispersed aerosol was generated with the FMAG and sampled for a duration of 3 minutes. A cotton swab was used to collect the particles on the impaction plate, and a second cotton swab was used on all the other surfaces of the AAS head. The particles downstream of the AAS head were collected on a fiberglass filter. Collected particles were dissolved in three separate 10 mL water solutions. A digital fluorometer was used to measure the fluorescence intensity (S) of each solution. The collection efficiency was calculated per Eq.1.

$$Efficiency = \frac{S_{impaction\ plate}}{S_{impaction\ plate} + S_{filter} + S_{other}} \qquad Eq.1$$

A second test method was utilized to atomize ammonium sulfate aerosol particle sizes smaller than 1 µm. A schematic of the test set-up is shown in Figure 3.

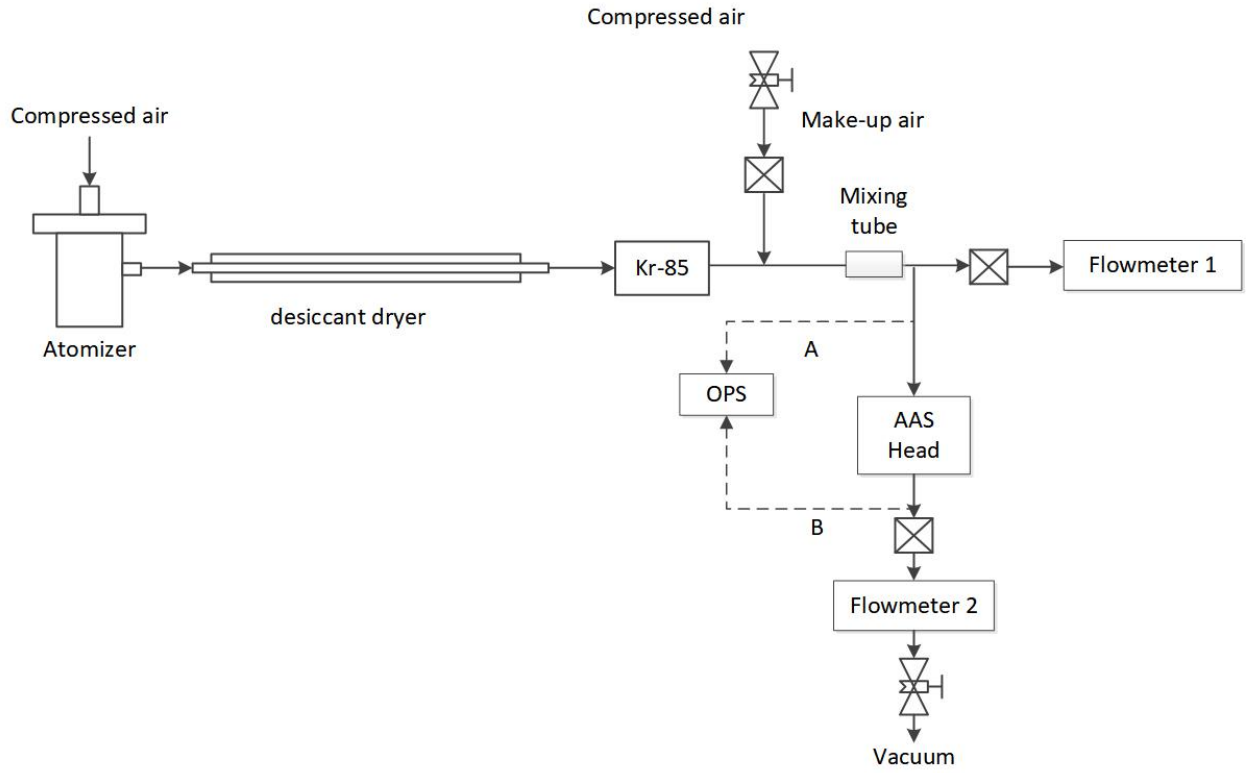

Figure 3: Test set-up for measuring physical efficiency with ammonium sulfate particles.

The make-up air and the vacuum were adjusted to sample from locations A and B with a TSI Optical Particle Sizer (OPS) Model 3330. This method of measurement provided upstream and downstream concentration measurements (C_A and C_B). This procedure was repeated multiple times to validate the repeatability of the test method.

ISO 14698-1:2000 defines the sampler efficiency as the test sampler count divided by the total count (from membrane sampler). Since particle counters measure airborne particles, it is difficult to directly measure the test sampler counts, so the measurement must be made indirectly. Looking at the test set-up, there are three places for particles to collect: the sampling medium, the walls of the sampler, and the filter downstream of the sampler. Therefore, in this test set-up:

Sampler Count = Total counts – counts on downstream filter – counts collected on sampler wall *Eq.* 2

Instead of counts, the OPS measures the particle concentration, so the equation for efficiency can be expressed as:

$$Efficiency = 1 - \frac{C_B}{C_A} - \frac{C_{sampler\ walls}}{C_A} \qquad Eq. 3$$

As long as the particle losses on the walls of the sampler are negligible, equation 2 can be simplified as:

$$Efficiency = 1 - \frac{C_B}{C_A} \qquad Eq. 4$$

Based on the results of the oleic acid tests, which measured particle collected on other surfaces of the sampler and showed these losses to be small (~2.5%), the collection efficiency was calculated using Eq. 4.

For the third test method, a test aerosol was generated within a test chamber with a HEPA-filtered air supply. A CAD model of this test set-up is shown in Figure 4. To avoid local concentrations of unmixed air, orifice plates were added upstream of the sampling points. OPCs (TSI OPC Model 7310-22005) were used to check the spatial concentration distribution to validate the suitability of this test chamber. Results showed spatial-uniformity uncertainty well within the repeatability of the particle counter concentration measurements.

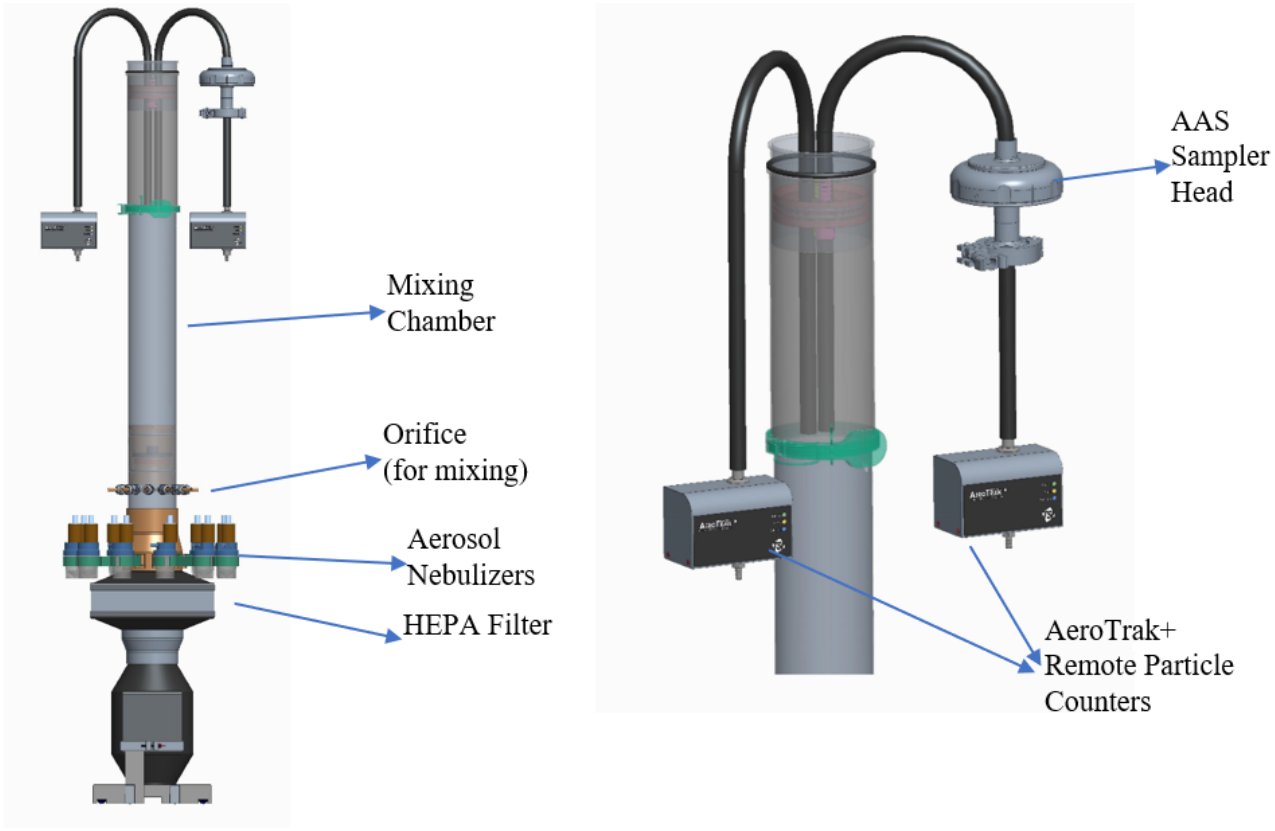

Figure 4: Test set-up for measuring physical efficiency with PSL particles.

During the tests, the airflow within the test chamber was maintained at 0.45 m/sec which is the flow rate in a typical cleanroom environment. The laboratory environment was maintained at 22°C and 45% humidity.

Per ISO 14698-1 section B.2.2.1, PSLs were used for this test. The aerosol was generated at the base of the mixing chamber and flowed upwards, mixed with dilution air driven by an impeller style duct fan (Figure 4). The nebulized PSL solution was pushed through mixing apertures before being sampled by iso-axial sampling probes connected to the particle counters. An initial run of measurements was completed to show agreement between the concentration values of the two particle counters without the presence of the AAS head. Once the concentration agreement was validated, the AAS head was added directly upstream of one of the particle counters. A plastic plate matching the dimensions of a filled agar plate, and coated with a silicon component to eliminate particle bounce from the hard surface, was placed inside the head. Various size PSLs were nebulized starting at 0.5 µm. The aerosol concentrations from the particle counter sampling from the mixing chamber (C_B) were compared to the particle counter concentrations downstream of the AAS (C_A). Again, the collection efficiency was calculated as follows in Eq. 5, since particle losses on other surfaces of the sampler are small.

$$Efficiency = 1 - \frac{C_B}{C_A} \qquad Eq. 5$$

Multiple sizes up to over 1µm were nebulized to generate a complete d_{50} value curve.

Experimental d_{50} Value Results

The results for the initial tests performed with oleic acid particles is summarized in Table 1. The results show that particles losses in the sampler average 2.5%, small enough to be ignored when calculating overall sampler efficiency using the alternate methods.

Table 1: Physical Efficiency of TSI AeroTrak+ Remote AAS using Oleic acid aerosols.

Aerodynamic Diameter (µm)	Physical Efficiency of AAS Head	Particles Collected in Filter	Particles Collected on AAS Walls
0.75	28.02%	70.59%	1.39%
0.95	88.95%	8.93%	2.12%
1.09	85.46%	11.5%	3.04%
1.54	94.97%	1.89%	3.14%

The results of all of the tests are summarized in Figure 5. The test results show that the AeroTrak+ Remote AAS has a d_{50} value at 0.8 µm, low enough to encompass many different organism types and clusters.

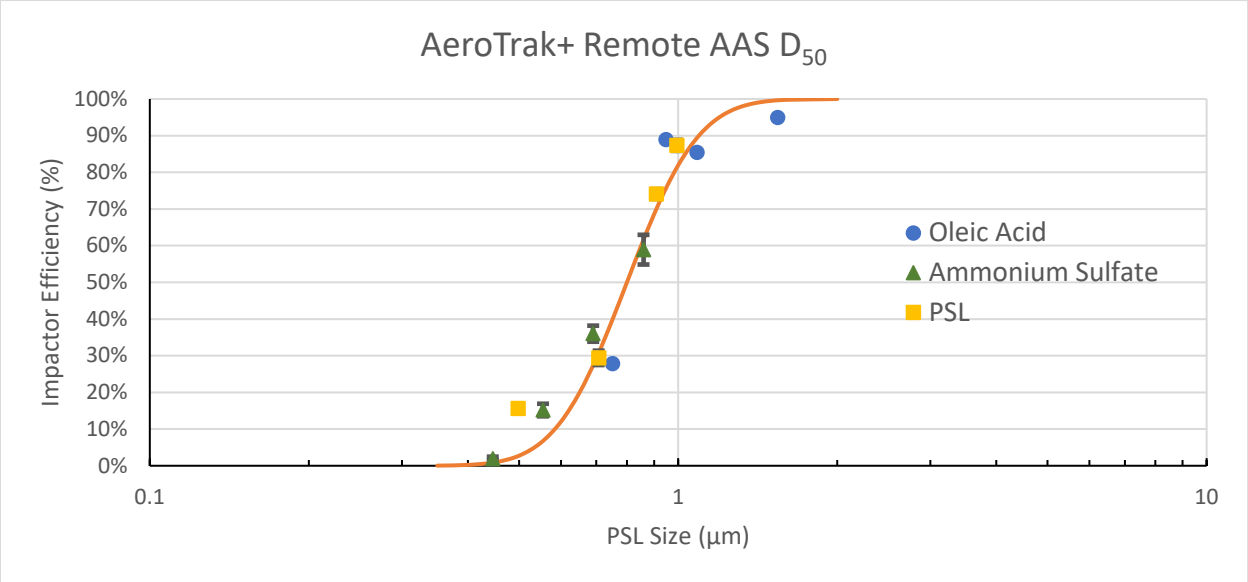

Figure 5: Physical efficiency data for TSI AeroTrak+ Remote AAS.

Conclusions—Meet the Needs of Pharma Aseptic Processing

The physical efficiency of the AeroTrak+ Remote AAS was tested using three different methods. The three different methods show good agreement, giving confidence in the test methodology. The results show a 0.8 µm d_{50} value, agreeing with the calculated d_{50} value of 0.89 µm. This d_{50} value is well below what is considered appropriate as per ISO 14698-1 and EN 17141. Because the tests with PSLs count a large number of particles, the counting statistics are very good. Hence, the resulting standard deviations were very small, with the graphical representation of the standard deviation on the smallest size of PSLs tested being smaller than the physical size of the data point on the graph. These standard deviations are much smaller than can be obtained by using bioaerosols, growing cultures, and then counting colonies, giving high confidence to the data obtained with this method.

Biological efficiency is dependent upon the sampler's physical efficiency, the ability of the collection media to support biological growth and the impaction velocity. Of these parameters, the physical efficiency and impaction velocity are controlled by the manufacturer of the sampler. The ability of the collection media to support biological growth is controlled by the user and their selection of media. TSI has optimized the biological efficiency of the AeroTrak+ Remote AAS by designing around an impaction velocity that balances particle collection (physical efficiency) and microorganism survivability.

As described earlier in this document, researchers have found that an impaction velocity between 30-50 m/s is desired for maximizing biological efficiency with a velocity near 40 m/s being ideal. The AeroTrak+ Remote AAS has an impaction velocity of 43 m/s, resulting in a low d_{50} value (0.8 µm), and an optimized biological efficiency. The results of biological efficiency testing using biological aerosols will be presented in a separate document.

With an impaction velocity which minimizes the effect of impact stress on microbial recovery and the optimum d_{50} value to capture microorganisms, the AeroTrak+ Remote AAS is well suited for viable air testing in aseptic cleanroom environments.

References

1. ISO 14698-1:2003, Cleanrooms and associated controlled environments – Biocontamination control – Part 1: General principles and methods
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3. Stewart, S. L., Grinshpun, S. A., Willeke, K., Terzieva, S., Ulevicius, V. and Donnelly, J. 1995. Effect of impact stress on microbial recovery on an agar surface. Appl. Environ. Microbiol. 61, 1232-1239.
4. Chen, M., Romay, F.J., Li, L., Naqwi, A., and Marple, V>A> 2016. A novel quartz crystal cascade impactor for real-time aerosol mass distribution measurement. Aerosol Science and Technology, 50:0, 971-983.

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