Application Note

Effective Low Volume Dispensing using BioJet Quanti and BioJet Plus Dispensers

Table Of Contents

Introduction

Robust dispensing of fluids in low nanoliter volumes is a technically demanding process involving multiple variables. This document is designed to help the user understand how to effectively and robustly dispense low nanoliter volumes using the BioJet Plus dispensing system.

The primary system components contributing to an accurate and reproducible low volume dispense are:

1. The dispenser
2. The environment
3. The fluids in the system
4. The substrate to be dispensed onto
5. Programming and process design factors
6. Dispenser cleaning and maintenance factors

Each of these system components will be discussed in detail in this document.

BIODOT

Section 1: The Dispenser

The BioJet Plus technology combines the high-resolution displacement capabilities of a syringe pump with a high-speed micro solenoid valve. This combination permits the non-contact dispensing of nanoliter volumes.

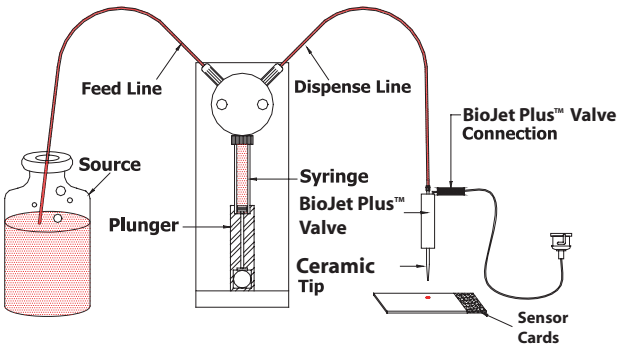


Figure 1 - BioJet Plus Dispensing System

In a typical dispensing system, 4 to 8 of these syringe/solenoid channels are placed together. Two modes of liquid handling are possible: Continuous (bulk) dispensing and aspirate/dispense.

Continuous dispensing involves pulling reagent or solvent from a reservoir into the syringe and then dispensing it through the micro solenoid valve. Filling the system with a backing fluid, dipping the tip of the valve into a sample, withdrawing the syringe to aspirate the sample, and then dispensing the aspirated sample accomplishes Aspirate/dispense.

The BioJet Plus™ dot dispensing system is a hydraulically driven system that requires a fluid medium to be present from the syringe to the microsolenoid BioJet Plus™ valve. The dispensing process involves the following steps:

1) The syringe is displaced a given amount
2) The valve is opened for a short period of time (milliseconds)
3) Fluid is released from the valve and travels to the tip
4) The fluid increases its linear velocity as it passes through the tip orifice and ejects as a drop (or stream if the amount of fluid is large). One valve actuation results in one drop.

The key to a correct volume being dispensed in a given drop from the BioJet Plus system is the steady-state pressure (SSP) in the dispensing system. This pressure has several important features:

- It is achieved by the displacement of fluid by the syringe pump
- The SSP is displacement (drop size) dependent, increasing with increasing displacement (drop size)
- The SSP is determined by the system compliance, which is dominated by entrapped air bubbles.
- Once the SSP is established, the amount of fluid displaced by the syringe pump will equal the amount dispensed

The BioJet Plus™ dot dispensing system can be modeled as an electrical circuit with the pressure acting as the voltage, the flow rate as the current, the system compliance as capacitive elements, the valve, tip, and feed lines as resistive elements, and the valve as a switch. This model shows the syringe pump as a current source, which provides an advantage over a pressure source (e.g. gas pressure) in that any changes in resistance will not affect the flow rate. In contrast a pressure source will be affected by changes in resistance in the system.

Factors Affecting the Dispense Volume

The model described above shows the fluidic circuit possessing a feedback loop, which can be used to achieve the SSP. Once the SSP has been achieved, the volume displaced will equal the volume dispensed.

The SSP is achieved by first pre-pressurizing the system by displacing an experimentally determined volume using the

syringe pump. Usually this pre-pressure is slightly higher than the required system pressure and requires several pre-dispenses at the desired volume to reach steady state pressure, whereby the desired dispense volume is dispensed.

Several factors influence the achievement and maintenance of the SSP and the desired dispense volume:

Priming

The Prime is used to initialize the syringe pumps and fill the syringe pumps, microsolenoid BioJet Plus valves of the dispense head, and connecting tubing with fluid from the reservoir(s). The reservoir fluid is either system fluid for aspirate/dispense of reagent, or sample fluid for continuous (line or dot) dispensing.

When the dispense system is primed, several hundred micro liters of fluid are dispensed as a stream. The resistance to flow caused by the valve and tip orifice causes the pressure within the system to become higher than desired for SSP. To achieve SSP, one must first vent the valves, which involves opening the valves without displacing fluid. This brings the system to ambient (zero) pressure and from this point; the SSP pressure can be achieved.

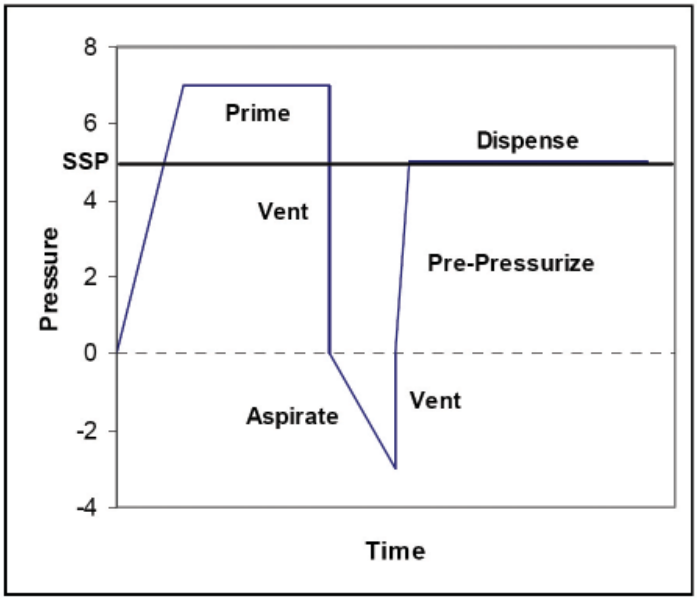
Aspiration

The aspirate function draws sample from a reservoir, usually a micro-well plate, into the tip of the dispense head. To perform an aspirate, several parameters must be set. These parameters are set by using Syringe Speed and Channel Parameter actions. The syringe speed controls the speed of the syringe pump dispensers. In general, slow syringe speeds are used for aspiration to prevent a large vacuum from being developed which can result in the development of bubbles in the system.

During the normal aspiration process, a slight negative pressure is produced. This negative pressure is relieved by performing a vent, which opens the microsolenoid valve without displacing any fluid. When sample is aspirated, the syringe pump draws fluid through the tip orifice. The resistance to flow from the tip and valve creates a negative pressure, which must first be overcome to achieve a steady state pressure.

As with priming, venting the system can bring the system to a known (zero/ambient) pressure, from which the SSP can be applied (Note: venting is typically done with tips in the sample to prevent the introduction of air).

The SSP during a prime/aspirate/dispense cycle is shown schematically below:



Gas Bubbles

Gas bubbles can occur in the dispensing system for multiple reasons, including primarily leaks and dissolved gases in the fluid. The major effect of bubbles is to change the system compliance, which can affect drop formation. Input will still equal output but the fluid will collect on the dispense tip rather than eject as a full drop. This can cause variation in dispensed volumes and eventually cessation of dispensing.

Bubbles can be removed by purging the system with isopropanol and using degassed solvents as well as the system fluid.

Tip Effects

Condensation of liquid on the dispense tips can lead to loss of control of dispense volumes due to the interaction of ejected drops with resident fluids near the tip orifice. This effect can be reduced and/or eliminated by vacuum drying of the tips at appropriate times during the dispense cycle. Vacuum drying the tips is especially important when dispensing for long periods of time. Tip orifice size is also of critical importance. Smaller drop sizes require smaller orifice sizes for proper drop formation. The smaller orifice size must, however, be balanced against the rheological properties of the fluid and the particulate content of the fluid to be dispensed, as smaller orifices clog more easily than larger orifices.

Syringe Speed

Syringe speed is the speed of displacement of the fluid in the syringe pump. For dispensing, the syringe speed has little if any affect on the SSP. For priming, the syringe speed will effect the pressure build-up and at very high speeds, may cause too much pressure and result in leaking. For aspiration, slow speeds are best to prevent excessive negative pressures being generated, which could also lead to out-gassing from the fluids.

Valve Open Time

The microsolenoid BioJet Plus valve open-time is one of the most important parameters for achieving and maintaining the SSP. This is the time the valve opens to release displaced fluid and eject the drop. In previous versions of software the open time is set in % duty cycle. Duty cycle gives the percent of time the valve is open for one valve actuation and the open-time is the actual amount of time that the valve is open in one valve actuation.

For a dispense system at SSP, the proper open time will result in a displaced volume equal to the dispensed volume. If the open time is too short, then over time pressure will build beyond the SSP. If the open time is too long, the SSP will be dissipated eventually resulting in drops not being ejected from the tip.

The open time is the approximate time required for the fluid to move though the valve. Thus open time increases with increasing drop size and increasing viscosity. A list of appropriate on-times is contained in the dispenser manual.

Section 2: Dispensing Environment

Many dispensing applications require careful control of the dispensing environment. This can entail control of :

- Humidity
- Static electricity
- Partial pressure of solvents or noble gases
- Airborne particulates

The use of environmental enclosures around machines can be critical in ensuring proper and continuous operation of the machine. The use of controlled, elevated humidity environments can be important in aspirate and dispense applications where open reservoirs of small volumes of aqueous reagents are used as the source for dispensing. Slightly elevated humidity can also help to reduce the buildup of static electricity which can be caused by the movement of certain substrates such as nitrocellulose through the unit.

Increased partial pressures of noble gases can be used to assist in reduction of oxidation of reagents, as well as to control drying, and increased partial pressures of solvents in the chamber can be used to reduce evaporation of solvent-based reagents within safe operating parameters

for the solvents (the possibility of flash must be particularly considered where use of this option is intended).

Critical to the continued trouble-free operation of the machine is minimizing the particulate content of the entire area in which the dispenser operates. Ideally, the unit should be operating in a Class 10,000 or better cleanroom with temperature and humidity control. At worst the machine should be enclosed to reduce the possibility of airborne contamination of the dispensing area with high levels of particulates which can serve to disrupt the operation of the BioJet valve.

Section 3: The fluid, including rheology, dissolved gases and particulate content

Fluid Rheology

Since there are constricted passageways within the BioJet Quanti3000™, the flow of liquid is influenced by both the viscosity and surface tension of the dispensed reagent. Fluids of viscosity up to approximately 10cP can be accurately dispensed, however for ideal results, fluid viscosity greater than 4 centipoise should be avoided since drop to drop variations are more likely to occur under these conditions. Highly compressible fluids or fluids demonstrating thixotropic properties must be used with care in the system. In aspirate and dispense applications, it is critical that the backing fluid used in the system be compatible with the fluid to be dispensed. The use of incompatible backing fluids can result in chemical reactions, leading to the formation of particulates, air bubbles, or filaments, any one of which is potentially disastrous to the application and to the BioJet valve. Certain fluids also contract on contact with water, leading to changes in volume that can affect the accuracy and reproducibility of dispensing.

Dissolved Gases

The presence of dissolved gases in the system can lead to inaccuracies in dispense volume or missed dispenses. Dissolved gases can come out of solution at virtually any point in the system that a nucleation site is available, which includes the inlet and outlet lines, the syringe, the three-port valve and the BioJet valve and tip. These bubbles may be macro-scale and be visible to the eye, or they may be microscopic or in a portion of the fluid pathway that is not visible to the operator, thereby making it extremely difficult to diagnose a dispensing issue when one arises. It is therefore critical to remove as much dissolved gas from the fluid as possible prior to use in the dispenser. This degassing can be achieved in a number of ways. BioDot recommends the use of helium degassing (described elsewhere in this document) but in cases where the required equipment is not available, a less efficient method of vacuum degassing may be used. All fluids that come into contact with the dispenser should be degassed and filtered, including backing fluids.

Particulate Content

BioJet valves operate in part through the motion of a poppet which is seated in a rubber seal. When the poppet is in the up position, the fluid pathway is open and fluids pass through the valve opening to the tip under pressure. When the poppet is in the seated position, the valve is closed and no fluid passes through to the tip. Correct seating of the poppet in the EPDM seal is one element of the operation of the system that ensures that the SSP is maintained, that no air enters the system, and that no fluid leaks from the system. If particulates become embedded in the rubber seal, they physically inhibit the proper seating of the poppet in the seal, thereby ensuring that all of the problems just described will occur. The presence of microscopic particulates in the valve seal is the number 1 cause of poor dispense quality and valve failure. Additionally, particulates can cause damage to syringe seals, 3-port valves, and can provide nucleation points for bubble formation in the system.

It is critical that all fluids used in the system be filtered before use, ideally with a 0.22um filter. This includes reagents, backing fluids and wash fluids. BioDot can provide in-line filters to reduce the particulate load drawn into the fluid pathway.

Chemical Compatibility

Aqueous Solvents

In general, aqueous solvents are compatible with the BioJet dispensing technology. Buffers with a pH range from 3 to 10 are suitable. Extremes in pH may cause corrosion of the stainless steel or glass materials. In addition, many surfactants and proteins can be used with the dispensing system.

Polar Organic Solvents

In general, polar oganic solvents are compatible with the dispensing system. Although not all polar organic solvents have been tested under long-term conditions, the following have shown good chemical compatibilty with the system:

- Dimethyl sulfoxide (DMSO)
- Acetone
- Methyl Ether Ketone (MEK)
- Ethyl acetate

Non-Polar Organic Solvents

Most non-polar organic solvents are incompatible with the Biojet dispensing technology. These solvents cause swelling and/or degradation of the polymeric parts of the microsolenoid valve, especially the EPDM seal.

Overcoming Chemical Incompatibility

In some cases, chemical incompatibilities can be overcome. For example, for samples dissolved in solvents which are not compatible with the standard BioJet valve, the sample can be aspirated up to but not into the valve (approximately 5 µL maximum aspiration volume). In cases where a larger aspiration volume is needed, a customized extension can be added between the valve and the tip. This results in an increase in the minimum dispense volume from 8 to 10 nL up to 50 to 100 nL. A second option is the use of a more inert valve. BioDot offers a more chemically inert valve, the performance of which can be assessed with the particular solvent of interest on an individual basis.

Section 4: The substrate

The physical characteristics of the substrate contribute a great deal to the quality and efficiency of a microdispense. Characteristics of the substrate that can compromise or contribute to a good quality result include:

- Hydrophobicity
- Planarity
- Reproducibility of modification (additives or treatments)
- Charge

Through careful application of the principles described in this document, the BioJet or BioJet Plus dispenser can deliver a quantifiable, reproducible, programmable volume from the tip of the dispenser. Quite often, what that dispense looks like once it hits the substrate will depend largely on the qualities of the substrate itself.

BioJets have been used to dispense on a wide variety of substrates, from glass slides to nitrocellulose, and a wide variety of plastics, both flexible and rigid. The quality of the dispense achieved can be measured in a variety of ways, and each application has different requirements for the aesthetics and volumetric accuracy to be achieved. Microarrays on glass slides or coated glass slides generally require a final spot with good morphology, perfect roundness and even density of deposition across the spot. The achievement of these characteristics depends on factors such as the hydrophobicity / hydrophilicity of the substrate, the (protein-) binding nature of the substrate, static charge, and wicking characteristics, as well as the rheological and

chemical characteristics of the fluid itself being dispensed. While it is relatively simple to optimize the volume of the dispense, it quite often requires more demanding work to optimize the interactions of the reagent, substrate and dispenser to achieve the aesthetic characteristics desired in the final spot. Other applications, such as many biosensor designs, are less demanding in terms of spot morphology after deposition, and demand more in terms of reproducibility of absolute dispense volume or coverage of a defined area at a defined thickness. These applications have their own demands in terms of surface characteristics of the substrate, particularly wicking or spreading characteristics, and tolerances on the placement of printed circuits. Variations in z-axis height on some substrates can cause issues with dispense placement accuracy, as consistent z-axis placement of the tip relative to the surface is important for ensuring accurate drop positioning.

Section 5: Programming and Process Design

5.1 Introduction

Great care should be taken during the initial stages of learning the control software for the BioJet and BioJet Plus systems. It is estimated that over 90% of the user-induced damage to BioDot systems occurs within the first week of operation at a customer site. These are sensitive robotic instruments, which, while robust enough to handle demanding manufacturing and R&D environments when handled correctly, can be severely damaged through incorrect operation. Common errors include:

- Crashing of heads through incorrect programming of the compound motion table or gantry
- Damage to BioJet or BioJet Plus valves through incorrect programming of valve operation
- Contamination of the system with particulates or incompatible fluids, leading to errors in dispensing accuracy or damage to valves.

It is strongly recommended that users purchase an Installation and Training package from BioDot, and / or utilize BioDot's Application Laboratory and technical staff to develop their programs and applications at least initially.

The User Manual for the unit should be carefully studied, and operators should pay particular attention to the sections on Cleaning and Programming of the BioJet or BioJet Plus dispensers.

Some basic principles should be applied to process design in order to achieve maximum system performance:

1. Pay particular attention to the geometry of the machine and the dispense head in designing the process in order to minimize process time and to create intelligent process designs
2. Determine early in the process design phase the optimal number of dispensers, balancing throughput, cost and process efficiencies
3. Consider fluid rheology and chemistry in determining backing fluids to be used
4. Consider solvent compatibility with the BioJet valves
5. Determine whether humidity and temperature control are required
6. Determine whether an increased atmospheric partial pressure of a solvent is required
7. Ensure that the backing fluid used is appropriate and compatible with the fluid to be dispensed
8. Think clean. Remove all sources of particulates in fluids, machine, disposables and parts to be dispensed
9. Use the sample programs provided with the machine to establish the basic program that you wish to use, then build complexity from there
10. Use sleep mode programs to prevent the need for continuous machine setup and teardown (see below)

5.2 In-Process Cleaning of Tips

Buildup of proteins on tips can lead to inconsistent dispensing due to clogging, and also to carryover of proteins from well-to-well of a source plate and array (if multiple samples are being aspirated in sequence using the same tip). Appropriate in-process cleaning is crucial to maintaining dispenser function.

Depending on the type of protein, hydrophobic interactions can mean that protein adheres quite strongly to the tip and removal of the protein from the tip can be difficult. Each system should be assessed separately, however as a general principal, the use of a buffered solution of MeOH (approximately 20%) plus 100mM NaCl, pH 7.4 (for example in Tris) will be adequate to disrupt binding of proteins to tips. Repeated immersion and aspiration of a cleaning solution such as this one, followed by rinsing with filtered distilled water should be sufficient to remove most proteins from the tips. This can be programmed as a periodic cleaning step in situations where a single sample is being dispensed repeatedly from a tip, or between samples.

5.3 Sleep Mode Programs

To maintain optimal system performance, it is advisable to minimize the number of times that the machine has to be shut down and restarted. Every time a shutdown and restart is performed, a cleaning and priming cycle is necessary, which often involves disconnection of syringes, tubing and valves, which in turn increases the opportunity for damage to components and can lead to increased opportunities for contaminants to enter the system. The use of Sleep Mode Programs is one way to minimize the number of setup and shutdown cycles. In this mode of operation, the system is left to idle when not in use, reactivating itself at intervals to dispense small amounts of innocuous fluids, cleaning fluids or backing fluids. The machine can then simply be reactivated when necessary, flushed and set up to run the required program without physical interference with the components of the fluid pathway.

A methodology for improving system performance through appropriate system setup, teardown, cleaning and programming is outlined below.

5.3.1 Purpose

To outline a procedure for setting up a machine in order to achieve consistent machine performance via degassing, filtering, use of helium and sleep mode.

5.3.2 Materials

- Any Biodot machine containing syringe pumps and BJQ's – configuration will include Minstac
- connections and ceramic tips
- Tank of helium
- Apparatus/manifold for connecting source reservoir to machine and helium tank concurrently
- Filter - at least 0.45µm but 0.2µm preferred. If in-line filters on the fluid inlet lines are to be used, a larger filter size (1µm) is recommended in order to minimize effects on the rapid achievement of Steady State Pressure in the system.

5.3.3 Methods

5.3.3.1 First time set-up

The first time a machine is used (OR anytime ANY hardware is changed), it must be assumed that the fluid path (or hardware) contains particulates that may cause the dispensing valve to fail (either mechanically or functionally). It must also be assumed that air is present in the fluid path which will greatly impede consistent dispensing. Therefore, anything coming into contact with the backing solution must first be cleaned, the backing solution, wash solution and dispensing solution must be filtered and the fluid path cleared of air. These things can be accomplished by cleaning removable parts offline, filtering, and flushing with ethanol, respectively.

Cleaning

Removable parts should be cleaned off-line (this will include the reservoir bottle, syringe and piston, in-line filter, if being used, and ceramic tip). Once cleaned, reattach all parts.

Filtering

Filtration of dispense fluids, ethanol, backing fluids and wash fluids can be performed off-line via a bottle-top filter or a syringe tip filter or in-line using BioDot's recommended in-line filter.

Flushing

Disconnect the Minstac fitting at the top of the dispensing valve(s) and position the end of the tubing over a waste basin – repeat this for all channels. Attach a reservoir bottle(s) containing filtered ethanol to the machine. Prime the ethanol through the fluid path until all visible air in the tubing is expelled and at least one more cycle – this will clean the tubing as well as remove any air in the fluid path.

Then reattach the Minstac fitting to the dispensing valve(s) and make sure the ceramic tip(s) are in place – repeat for all channels. To remove air from the dispensing valve(s) and tip(s), prime through with 2 more cycles of ethanol. The ethanol then needs to be removed from the fluid path which can be done by priming de-gassed backing solution (see below for procedure) through all lines. For most BioDot machines, 10 prime cycles will suffice to remove all the ethanol.

Degassing

All fluids entering the fluid pathway must be degassed. Below are two procedures for de-gassing using vacuum and helium.

Vacuum Degassing

- Using BioDot's Reagent Degasser DG950, de-gas the reservoir bottle(s) using sonication for 10 minutes – sonication can be run in 10 second intervals.
- If this equipment is not available, apply a vacuum to the reservoir bottle(s) for at least 10 minutes.
- When the de-gassing is complete, **DO NOT** pour the liquid into another container as this will redissolve air into the liquid. Cover the bottle with a cap or wrap tightly with parafilm to transfer the bottle to the machine.

Helium Degassing

- Note: This procedure must be performed with BioDot's helium degassing system. It is designed for use with fluids to be bulk dispensed (ie put through the fluid pathway, not aspirated) or with backing fluids for aspirated fluids.
- Fill reservoir bottle(s) with desired solution.
- Apply 15 psig of helium to bottle(s) for 30 minutes. Vent to 0.1 psig.
- Repeat step 2 twice for a total of 3 fill and vent cycles.
- Maintain an operating pressure of 0.1-0.2 psig helium for dispensing

5.3.3.2 Machine Set-Up (Not First Time)

Once the machine has been set-up according to the above outline, a clean and de-gassed state may be maintained by 1) not changing hardware and 2) always maintaining a constant pressure of helium (i.e. the operating pressure). This means neither the machine nor helium will ever be turned off. When the reservoir needs re-filling, the helium will need to be turned off so the set-up procedure will begin at Filtering above.

5.4 Sleep Mode

Good quality, consistent dispensing requires great attention to detail when setting the machine up for operation. The factors above, achieving and maintaining a de-gassed backing solution, filtering, cleaning and flushing, must all be addressed each and every time the machine is to be used. In order to minimize the need to perform the above procedure, the machine should be placed in a "sleep mode" when not in use. An example of a Sleep Mode program is outlined below:

- Move to Wash – places tips down into wash water.
- Pause – Fixed duration of 15 minutes (900,000ms)
- Move – lift tips out of wash water
- Vacuum Dry Tips
- Move to Waste
- Prime – 1 cycle (be sure to check for sufficient backing solution and to set appropriate syringe speeds)
- Vacuum Dry Tips
- Move to Waste
- Loop 50X - 100nL dispenses (be sure to include a syringe speed – 10-20-1000- and open time – 1500ms)
- Prime - 1 cycle

This entire string should be within 1 function and that function looped according to the following formula → # hours machine will be unused * 60/15 minutes = # of time to loop function (or # of 15 minute cycles in total downtime). The "sleep mode" program then is simply this one function looped x # of times.

Section 6: Dispenser Maintenance

The BioJet and BioJet Plus dispensing systems consist of three major components:

- Controller
- XYZ motion (compound motion table or gantry)
- Fluid pathway

The maintenance requirements of the controller and XYZ motion system are minimal and these components of the system rarely generate problems in terms of the quality of product produced. The fluid pathway is the portion of the system that requires the most care and attention, constant cleaning, and occasional maintenance and part replacement.

The fluid pathway consists of:

- Fluid reservoir (or source plate)
- Inlet tubing to the 3-port valve
- 3-port valve
- Syringe
- Outlet tubing from the 3-port valve
- BioJet valve
- Tip

All portions of this pathway are considered to be disposables and are subject to reduced warranty periods, so constant care must be taken to ensure long life and appropriate performance.

In order to maximize life and minimize problems with dispense accuracy, several basic principles should be adhered to:

1. Filter all fluids in the system, ideally with a 0.22um filter before use. This includes backing fluids, wash station fluids and fluids to be dispensed. If possible, use in-line filters for fluids to be bulk-dispensed (i.e. not aspirated).
2. Degas all fluids, ideally using helium degassing, or using vacuum with intermittent agitation or sonication if helium is not available.
3. Flush lines with alcohol regularly to reduce bubble formation
4. Work in a particulate-free environment. Ideally a class 10,000 or better cleanroom should be used. Remove particulates from reservoirs and machine before use

5. Rigorously and diligently follow all recommended cleaning procedures (see Appendix)
6. Check performance and be prepared to replace ALL fluid pathway components on a regular schedule
7. Use only compatible fluids within the BioJet valve (see Appendix for list of known incompatible fluids). All other fluids should be aspirated and dispensed from the tip and not put through the valve itself
8. Check performance of the system regularly using a calibrated measurement system such as the Artel PCS (see Appendix for details)
9. Utilize “Sleep Mode” programs (see Process Design section. Never turn off the machine if possible
10. Clean tubing separately from BioJet valves. If particulates accumulate in tubing they can be washed into the valve during cleaning of the system. Disconnect the tubing from the BioJet valves before performing cleaning of the tubing. Subsequently reconnect the tubing and clean the valves.

Summary: Considerations for Effective Nanoliter Dispensing

Think clean
Remove all possible sources of particulates in the environment, fluids, machine, disposables and substrates

Work in a particulate-free environment
Where possible a class 10,000 cleanroom

Remove particulates from reservoirs and machine before use
Foreign particles can damage both the 3 port valves and the solenoids.

Filter all fluids in the system before use
Including backing fluids, wash station fluids and dispensed fluids. In-line filters can also be used.

Degas all fluids
Ideally use helium degassing, or vacuum with intermittent agitation or sonication.

Follow BioDot’s cleaning procedures
Use BioDot-specified cleaning materials and reagents.

Clean tubing separately from valves
Disconnect the tubing from the BioJet valves before cleaning the tubing to prevent contaminants entering the valve.

Flush lines with alcohol regularly
To reduce bubble entrapment in the fluid path.

Check performance of the system regularly using a calibrated measurement system
Use a system such as the Artel PCS or a validated microtiter plate – based method

Replace ALL fluid pathway components on a regular schedule
This includes:

- Fluid reservoir (or source plate)
- Inlet tubing to the 3-port valve
- 3-port valve
- Syringe
- Outlet tubing from the 3-port valve
- BioJet valve
- Tip

Use only fluids compatible with the valve
All other fluids should be aspirated and dispensed and not put through the valve itself

Maintain the stability and uniformity of your reagents
Operate in a temperature controlled environment or incorporate mixers, heating or cooling plates into the machine design

Choose source plates or reservoirs appropriate to the

application. Consider:

- Protein binding characteristics
- Aspect ratio
- Capacity

Choose the correct backing fluid for your application

Consider fluid rheology and chemistry in determining backing fluids to be used

Consider the physical characteristics of the substrate, including:

- Hydrophobicity
- Planarity
- Uniformity of modification
- Charge

Maintain careful control of the dispensing environment, including:

- Humidity
- Static electricity
- Partial pressure of solvents or noble gases
- Airborne particulates

Take care in programming

Use the sample programs provided with the machine to establish the basic program to be used, then build complexity from there.

Never turn off the machine if it can be avoided

Utilize “Sleep Mode” programs to minimize teardown and setup, which increase the likelihood of damage, introduction of foreign particles and air into the system.

Take care of your dispense head and tips during program development

Crashing of heads during program development is the main source of damage to heads and tips.

Prevent cross contamination of wells in source plates

Clean tips carefully between aspirations. Ensure that the entire tip length exposed to the aspirate is washed before the next aspiration to prevent carry-over of fluids. Use cleaning solutions appropriate to the reagents being handled.

Appendix

1. Cleaning of the BioJet Dispensing System

General Notes:

- All cleaning fluids must be filtered and degassed
- The cleaning method outlined here is valid for bulk dispensing of aqueous, proteinaceous reagents. It is a suggested protocol only. Users should validate their own protocol designed for use with their own particular application and reagents.
- If aspiration of multiple reagents using the same tip is being performed it is critical that the cleaning process be validated to ensure that no carryover of reagent from well to well occurs. It is likely that a solution such as MeOH (approximately 20%) plus 100mM NaCl, pH 7.4 (for example in Tris) will be adequate to disrupt binding of proteins to tips, but depending on the backing solution solvent used, this may require change and validation.
- The cleaning protocols outlined are suggested for use in systems where Sleep Mode programs are not used.

Daily Cleaning

To achieve optimum performance and maximum life from the BioJet Quanti3000™ dispenser, it is recommended that the routine cleaning procedure listed below be followed after each period of use (at least once daily).

1. Purge supply lines of reagent.
2. Clean and refill the supply reservoir with deionized water containing 0.05% BioTerge to enhance scrubbing of interior recesses within the BioJet Quanti™.
3. Prime dispenser for 5 syringe cycles.

4. Repeat steps 1 through 3 with deionized water.

The reagent reservoirs and feed lines are reusable and may be used for extended periods of time before replacement is warranted.

Residue Cleaning (Weekly Cleaning during heavy use)
After prolonged dispensing of reagents, some buildup of protein constituents, salts, latex materials, or other particulate matter may occur. It is recommended that the following cleaning steps be followed on a weekly basis to dissolve any accumulated materials. This should be performed in addition to the daily cleaning procedure.

Option 1

1. Purge supply lines of fluids.
2. Clean and refill the supply reservoir with a dilute base (0.1N NaOH). Prime the BioJet Quanti™ for 5 syringe cycles and allow to sit for 10 minutes.
3. Prime using deionized water as in steps 1 and 2 above.
4. Clean and refill the supply reservoir with a dilute acid (0.1N HCl). Prime the BioJet Quanti™ for 5 syringe cycles and allow to sit for 10 minutes.

5 Purge the supply lines and prime for a minimum of 10 cycles using deionized water.

Option 2
For use in systems dispensing proteinaceous fluids or where acid/base use is undesirable

This procedure may be performed as part of the regular weekly cleaning protocol in addition to Option 1 above or as an alternative if the use of acid / base is undesirable in your system. It is of particular value in

helping to prevent cross-contamination when dispensing multiple reagents from a single BioJet Quanti3000™, or to clean protein buildup from the lines and the valve.

1) Purge supply lines of reagent
2) Prime 3 cycles of deionised water with 0.05%. BioTerge through the system.
3) Prime this solution out of the system.
4) Prime Jetwash into the system and allow to sit in the BioJets for 30 minutes.
5) Prime this solution out of the system.
6) Clean and refill the supply reservoir with deionized water.
7) Prime dispenser for 5-10 syringe cycles.
8) Remove the glass syringe from the syringe pump.
9) Remove the plunger from the syringe and flush deionized water through the open syringe and wash the plunger in deionized water being careful not to damage the plunger seal.
10) Flush deionized water through the ports in the 3-port valve. The valve stem may be turned by hand to switch path positions.

Cleaning of tips
Ceramic tips may be removed from the dispenser and washed in 1-10N acid followed by an equal molarity base, in turn followed by flushing with water. Appropriate safety precautions should be observed when handling high molarity acids and bases.

2. Chemical Compatibility with the BioJet Dispensing Technology

Introduction
The BioJet™ dispensing technology, can be used with a wide variety of solvents and reagents. However, there exist a limited number of solvents and reagents that can potentially damage the system. This application note examines the extent of chemical compatibility of a system using the BioJet dispensing technology.

Wetted Materials

The materials which come in contact with fluids in the dispensing system are listed below:

Syringe Pump

- Teflon
- Glass
- Stainless Steel

Microsolenoid Valve

- Stainless Steel (430F, 316SS)
- Polyphenylene Sulfide (PPS)
- Poly Ketone
- Ethylene/Propylene Diene Mono (EPDM)
- Epoxy (minimal wetted area)

Other Parts

- Teflon (transfer lines)
- PEEK (fittings)
- Polypropylene or Ceramic (dispensing tip)

For continuous dispensing, the fluid wets all of the above materials. For aspirate/dispense, the fluid comes in contact with the dispensing tip (aspiration volume < 6 µL) and the microsolenoid valve (aspiration volume > 6 µL)

Chemical Compatibility

Aqueous Solvents

In general, aqueous solvents are compatible with the BioJet dispensing technology. Buffers with a pH range from 3 to 10 are suitable. Extremes in pH may cause corrosion of the stainless steel or glass materials. In addition, many surfactants and proteins can be used with the dispensing system.

Polar Organic Solvents

In general, polar oganic solvents are compatible with the dispensing system. Although not all polar organic solvents have been tested under long-term conditions, the following have shown good chemical compatibilty with the system:

- Dimethyl sulfoxide (DMSO)
- Acetone
- Methyl Ether Ketone (MEK)
- Ethyl acetate

Non-Polar Organic Solvents

Most non-polar organic solvents are incompatible with the Biojet dispensing technology. These solvents cause swelling and/or degradation of the polymeric parts of the microsolenoid valve, especially the EPDM seal.

Overcoming Chemical Incompatibility

In some cases, chemical incompatibilities can be overcome. For example, for samples dissolved in solvents which are not compatible with the microsolenoid valve, the sample can be aspirated up to but not into the valve (approximately 5 µL maximum aspiration volume). In cases where a larger aspiration volume is needed, a customized extension can be added between the valve and the tip. This results in an increase in the minimum dispense volume from 8 to 10 nL up to 50 to 100 nL.

Summary

The following table shows the compatibility of various solvents and reagents:

	Compatible
Aqueous	
Water	Yes
PBS	Yes
Buffers pH 3 to 10	Yes
BSA	Yes
Surfactants	Yes
Polar Organics	
Acetone	Yes
Ethyl Acetate	Yes
Methyl Ethyl Ketone	Yes
Dimethyl Sulfoxide (DMSO)	Yes
Isopropanol	Yes, short term*
Methanol	Yes, short term*
Non-Polar Organics	
Hexane	No
Toluene	No
Methylene Chloride	No

*less than 1 hour

For specific solvents not listed here, please inquire as to possible testing of the solvent.

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