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Design and Evaluation of an Automation-Compatible Multiwell™ Insert for Cell-Based Assays

Doug Henderson*, Project Engineer and Darwin Asa, Ph.D.†, Senior Product Development Scientist

*BD Biosciences, One Becton Drive, Franklin Lakes, NJ 07417

†BD Biosciences, Two Oak Park, Bedford, MA 01730

Introduction

In recent years, the use of cell-based assays for drug discovery screening has increased tremendously, but some cell-based assays remain difficult to automate due either to special cell handling conditions or to general format incompatibility with robots. This is particularly true of membrane-based cell assays where separate housings (or inserts) support individual membranes. These inserts are difficult to manipulate, making automation complicated and expensive. To resolve this problem, BD Biosciences has developed an automation-compatible 24-well insert system and we have tested the system in several cell-based applications. The following data shows many of the design features and performance characteristics.

Materials and Methods

Design

To design an automation-compatible Multiwell Insert System suitable for use in membrane-based cell assays, it was necessary to

optimize for many of the following characteristics:

- Ease of set-up or use
- Ease of robotic manipulation
- Fluid handler access to the upper and lower chambers for sampling
- Prevent cross-contamination between chambers
- Consistent performance in membrane-based assays

This was done using an iterative procedure and Autodesk's Mechanical Desktop® installed on a desktop PC. Manufacturing equipment was then constructed and the insert system components were made.

Evaluation

The system was designed and tested for several physical performance characteristics (Figures 1-4) as well as several cell-based assays (Figures 5):

Caco-2 Assay (1 µm PET membrane) - A 24-well Multiwell Insert System with a 1 µm PET membrane was coated with fibrillar collagen. Caco-2 cells were seeded onto these coated inserts using Basal Seeding Medium supplemented with Mito+ Serum Extender (BD Biosciences). Cells were cultured for 24 hours and then the medium was removed and changed to Entero- STIM Medium supplemented with Mito+ Serum Extender. Cells were cultured for another 48 hours. The medium was then removed and Caco-2 barrier function was assessed by Mannitol permeability measurement.

Neutrophil Chemotaxis Assay (3 µm PET membrane) - Human neutrophils, obtained from healthy donor whole blood using progressive dextran sedimentation and Ficoll gradient procedures, were added to a 24-well Multiwell Insert Plate with a 3 µm PET membrane in a 24-well plate (BD Biosciences). Buffer containing 1×10^{-8} M fMLP (f-Met-Leu-Phe) was used as a chemoattractant in the lower chamber. Neutrophils were allowed to migrate for 90 minutes. At that time cells were fixed, stained, and counted by microscope to determine % cell migration.

Tumor Cell Invasion Assay (8 µm PET membrane) - A 24-well Multiwell Insert System with an 8 µm PET membrane was coated with BD Matrigel™ Basement Membrane Matrix (BD Biosciences) and then rehydrated with DMEM (Dulbecco's Modified Eagle's Media) for 2 hours at room temperature prior to use. After rehydration, media was removed and either 3T3 or HT 1080 cells were added to the top of each insert chamber. Using 3T3 conditioned media as a chemoattractant, cells were incubated for 24-hours. After incubation, non-invading cells were carefully removed from each insert with a cotton swab. Migrated cells were fixed, stained, and photographed microscopically to determine the extent of migration.

Results and Discussion

As shown in **Figures 1 and 2**, the BD Falcon™ HTS Multiwell Insert System is composed of a PET (polyethylene terephthalate) Multiwell Insert Plate, Feeder Tray, and non-directional Lid. This material was chosen for its excellent cell compatibility and optical characteristics. The Insert Plate is designed for use with the standard BD Falcon 24-well plate.

As can be seen in **Figure 3**, this system has many features designed to facilitate automation. Twenty-four inserts were integrated into a one-piece design to facilitate manipulation. Flanges on each component have at least 6 mm vertical gripping surface. The Insert Plate flange can be gripped with the lid on and is a readable surface for labeling or identification. Further, the inner edge of this flange is beveled to aid alignment during assembly. The Lid is rectangular, free of interior features, smooth on top for vacuum delidders, and has minimal system overhang. The assembled system height is 2 mm higher than a BD Falcon 24-well plate and lid.

Figure 3 also shows vertical distances and X-Y locations of the sampling port and well centerlines, referenced from the A1 corner. The D-shaped flat-sided Insert wall provides a 4.0 mm sampling port width. This generous port permits fluid handler access to the lower chamber using up to 1000 µl pipet tips without removing the Insert Plate.

Figure 4 provides some performance characteristics of the BD Falcon HTS Multiwell Insert System. Contamination from capillary action between upper and lower chambers is minimized by the addition of concentric rings to the lower outside of each insert wall. Feeder Tray ribs, originally designed as side wall supports, were observed to reduce liquid sloshing during system movement. Assay liquid volumes, procedural steps, and durations were used as a baseline to determine final component sizes, membrane seal requirements, etc. Liquid working volumes were then maximized to eliminate process steps such as cell feeding, which resulted in more robust assays.

Figure 5 demonstrates the use of the BD Falcon HTS Multiwell Insert System in several cell-based assays. These systems performed as expected in both cell barrier and cell migration assays and were equivalent to appropriate individual insert controls. Specifically, BD Falcon HTS Multiwell Insert Systems supported Caco-2 cell barrier formation and migration of either neutrophils or tumor cells (HT 1080).

Conclusion

We have demonstrated the utility and performance characteristics of the BD Falcon HTS Multiwell Insert System in several cell-based assays. We believe this system provides a means to automate many commonly used membrane-based cell assays and increase the efficiency, productivity and throughput of these assays in the drug discovery process.

BD Falcon HTS Multiwell Insert System

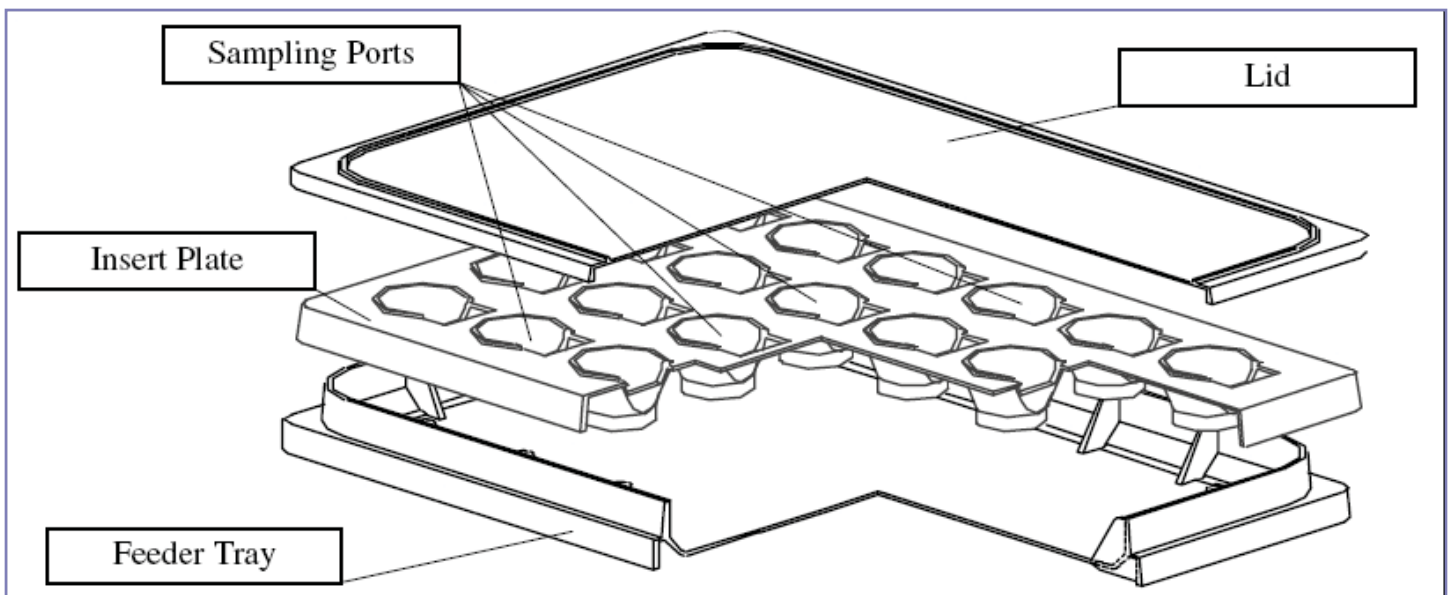


Figure 1: The BD Falcon HTS Multiwell Insert System features generous access ports and extended insert plate flanges for easy handling by robotic equipment, with or without the lid in place, a Feeder Tray to assist in uniform cell culturing, and a non-directional lid for easy lid placement.

BD Falcon™ HTS Multiwell Insert System Basic Dimensions

LID:

Material: PET (Polyethylene Terephthalate)
 Length: A = 129.57 mm (5.101 inches)
 Width: B = 86.82 mm (3.418 inches)
 Height: C = 8.20 mm (0.323 inches)

INSERT PLATE HOUSING:

Material: PET (Polyethylene Terephthalate)
 Length: D = 127.61 mm (5.024 inches)
 Width: E = 85.01 mm (3.347 inches)
 Height: F = 18.14 mm (0.714 inches)

Insert Well:

Top Interior Diameter: 12.50 mm (0.492 inches)
 Bot. Int. (Membrane) Dia: 6.50 mm (0.256 inches)
 Bot. Ext. Diameter: 10.00 mm (0.394 inches)
 Total Well Depth: 18.14 mm (0.714 inches)
 Well to Well Distance: 19.30 mm (0.760 inches)
 Sampling Port Length: 9.50 mm (0.37 inches)
 Sampling Port Width: 4.00 mm (0.16 inches)

FEEDER TRAY:

Material: PS (Polystyrene)
 Length: G = 127.86 mm (5.034 inches)
 Width: H = 85.47 mm (3.365 inches)
 Height: I = 19.94 mm (0.785 inches)

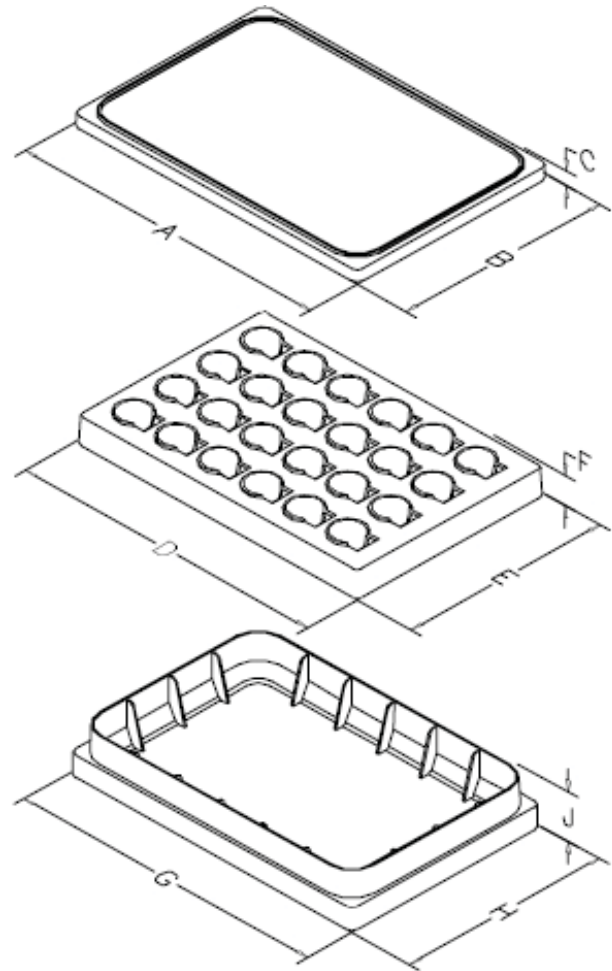


Figure 2: Composed of three components, the BD Falcon HTS Multiwell Insert System has been designed to be compatible with common laboratory automation. Basic dimensions are shown above.

BD Falcon HTS Multiwell Insert System Programming Dimensions

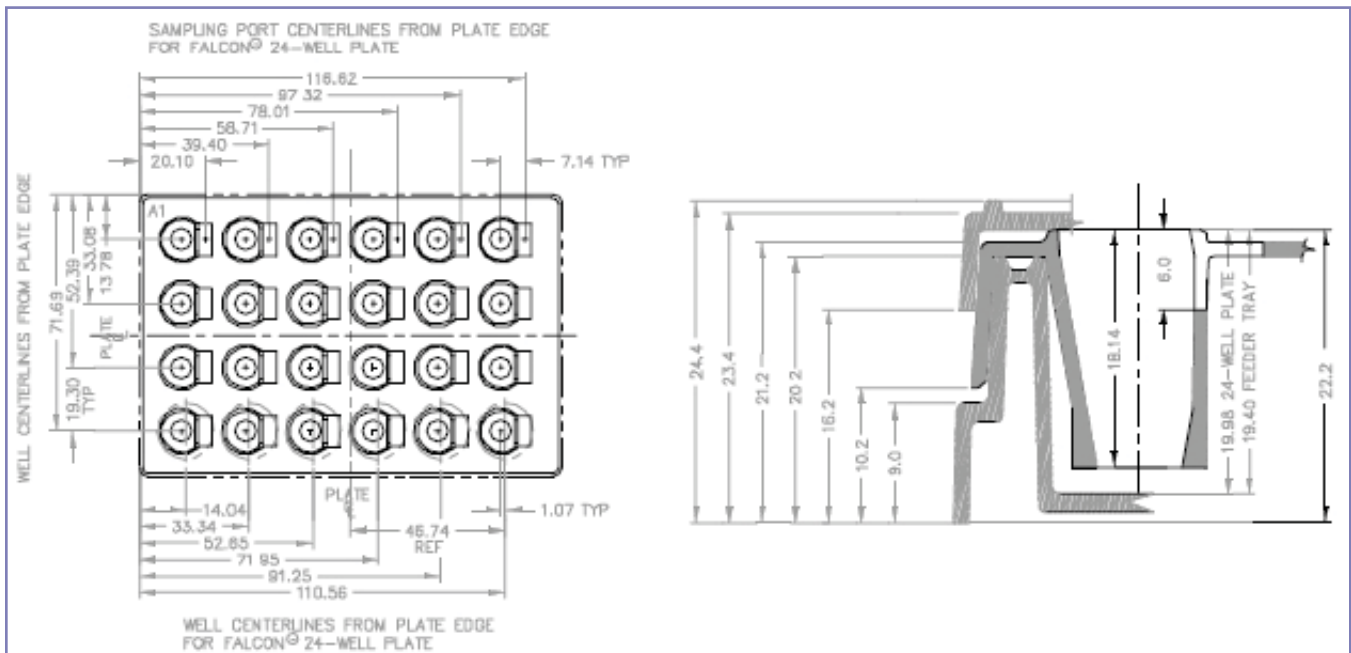


Figure 3: The BD Falcon HTS Multiwell Insert System has been designed to allow for optimal access to both upper and lower chambers. A side skirt suitable for automation is included in the design. The BD Falcon HTS Multiwell Insert System is also designed such that lot components can be manipulated either as a unit or by any combination of sub-components.

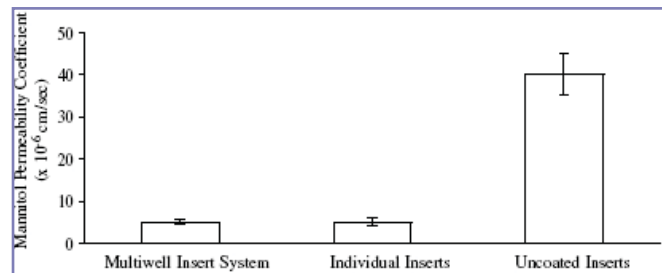
BD Falcon™ HTS Multiwell Insert System Performance Characteristics

Performance Characteristics	Tested Parameters	
Membrane seal durable for typical assay time periods	Membrane seal integrity maintained in media at incubation temperatures	Seal maintained for more than 3 weeks
Insert chamber holds appropriate media volume for assays	Cross contamination of upper/lower chamber	Insert chamber holds up to 500 µl without spill-over
No wicking of liquid from bottom chamber into insert chamber	Cross contamination of upper/lower chamber	Feeder tray holds up to 35 ml and 24-well plate holds up to 1.4 ml in each well without wicking
System holds liquid for cell growth cell assays without spillage upon agitation	Cross contamination of upper/lower chamber with agitation	Agitation up to 100 rpm possible without cross contamination with 0.5/1.4 mls in upper/lower chambers
System compatible with Endohm meter for measuring electrical resistance	Fit with WPI Endohm Multiwell Ohmmeter	Compatible with Endohm meter
Good Stacking	Deviation from 90°	<2° lean with a stack of 10 systems
System maintains sterile environment suitable for conducting assays	Presence of bacteria, fungi, mycoplasma	Tested and found negative
Access to upper/lower chambers	Access by 200 & 1000 µl pipet tips, and 1 ml, 5 ml, 10 ml, 25 ml pipets	Able to dispense and aspirate top & bottom chamber with 200 & 1000 µl pipet tips, and 1 & 5 ml pipets. Able to dispense with 10 and 25 ml pipets

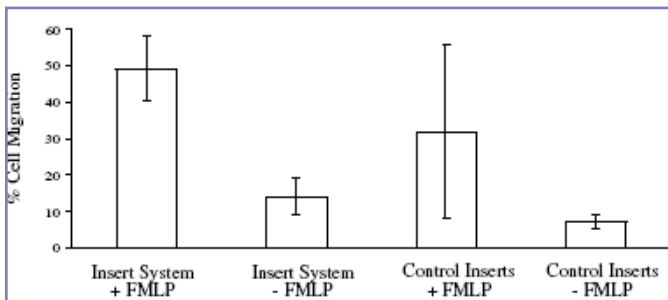
Figure 4: Several performance characteristics of the system were determined, using various methods. All tests involving liquid were prepared with appropriate assay or cell culture media.

Performance of the BD Falcon HTS Multiwell Insert System in Cell-Based Assays

Caco-2 Mannitol Permeability Assay



Neutrophil Chemotaxis Assay



Tumor Cell Invasion Assay

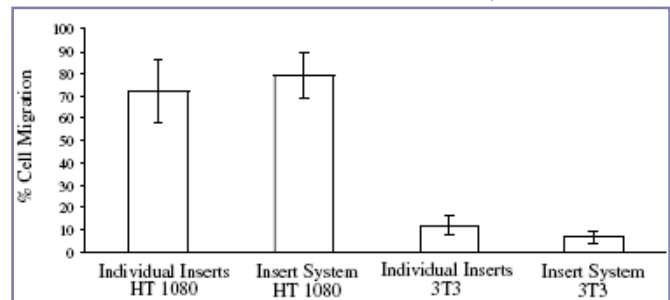


Figure 5: The BD Falcon HTS Multiwell Insert Systems (1, 3, and 8 µm PET membranes) were used in several cell-based assays as described above. BD Falcon HTS Multiwell Insert Systems performed as expected and compared favorably to assays performed in comparable individual BD Falcon inserts.

BD Biosciences
Two Oak Park
Bedford, MA 01730 USA
tel: 877.232.8995
fax: 800.325.9637

BD
Akasaka Garden City,
Akasaka 4-15-1, Minato-ku,
Tokyo, 107-0052 Japan
tel: (81) 24 593 5405
fax: (81) 24 593 5761

BD Biosciences
Erembodegem-Dorp 86
9320 Erembodegem, Belgium
tel: (32) 53 720 211
fax: (32) 53 720 450
contact_bdb@europe.bd.com

BD
2280 Argentia Road
Mississauga, Ontario
Canada L5N 6H8
tel: 866.979.9408
fax: 800.565.0897

BD Biosciences
Singapore Branch
30 Tuas Avenue 2
Singapore 639461
tel: (65) 6861 0633
fax: (65) 6860 1590

BD Biosciences
4 Research Park Drive
Macquarie University Research Park
North Ryde NSW 2113 Australia
tel: (800) 656 100
fax: (612) 8875 7200
aus_customerservice@bd.com