Thermo Scientific Sensititre Susceptibility Plates: Inoculation and Aerosol Containment Study

Application Notes

Key Words

aerosols, inoculation, incubation, mycobacteria, decontamination, Mycobacterium tuberculosis, Serratia marcescens, Mycobacterium fortuitum, Sensititre AIM Automated Inoculation Delivery System, pipette

Goal

To evaluate aerosol risk associated with the inoculation and incubation of a Thermo Scientific Sensititre Mycobacterium tuberculosis (Part No. MYCOTB) microtitre plate.

Background

The Thermo Scientific Sensititre AutoInoculator (Part No. V3010) and manual multi-channel pipette are used by microbiology laboratories throughout the world to inoculate plates for bacterial susceptibility and identification testing. The Sensititre product line now includes a new automated inoculation instrument, the Thermo Scientific Sensititre AIM Automated Inoculation Delivery System (Part No. V3020). All Sensititre inoculation instruments have been designed for use by experienced technologists trained in good laboratory practice. When following the recommended protocols provided with Sensititre™ instrumentation, and with adequate training, a microbiologist is at little risk when testing specimens that are processed in a Biosafety Level 2 (BSL-2) work environment. Microbiologists are also recommended to follow their site's standard operating procedures (SOPs) related to mycobacteria testing.

The Sensititre product line recently launched a plate for the susceptibility testing of *Mycobacterium tuberculosis* (Part No. MYCOTBI**), which belongs to the *Mycobacterium tuberculosis* complex and is classified as a BSL-3 organism. Working with *Mycobacterium tuberculosis* complex organisms in the laboratory requires a containment area using a biosafety cabinet (BSC). Currently, the inoculation of the MYCOTB plates from a standardized inoculum is performed using a multi-channel pipette and an open seed tray reservoir, which can pose some safety issues for laboratories working with mycobacteria.

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There have been no studies on aerosol generation using the Sensititre AIM™ System or a multi-channel pipette. Therefore, a series of experiments were conducted to verify that the recommended inoculation methods for Sensititre products are safe when microbiologists follow standard laboratory procedures associated with BSL3-rated isolates.

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Objective

The objective of the study was to evaluate aerosol risk associated with the inoculation and incubation of a MYCOTB plate. Two inoculation methods were evaluated; the multi-channel pipette and the Sensititre AIM System. Additional studies were performed to document the effectiveness of decontaminating the plate after inoculation and sealing procedures, to determine the effectiveness of the plate seal to contain liquid in an inoculated plate. The highest potential for risk of aerosols is during the plate inoculation phase; therefore, this study was performed to assess the risk and adequacy of decontamination procedures.

Methods

This study was designed to determine the extent of aerosol risk using two different inoculation methods. A 0.5 McFarland (1.5 X 10^8 CFU/mL) was prepared from a culture of pigmented *Serratia marcescens* and *Mycobacterium fortuitum* isolates and inoculated into blank Sensititre plates. After inoculation, the surrounding workspace area was sampled in a grid pattern to determine the extent of aerosol generation in the adjacent work areas. The plate surface (before and after a seal was applied) and the Sensititre AIM instrument were also sampled for aerosol contamination for the *Serratia* isolate, only. The effectiveness of the recommended plate decontamination procedure was determined by inoculating the plates, sealing them, and then disinfecting the plates before sampling took place. To determine the integrity of the plate seal to contain the liquid within the plate, plates were dropped from twelve inches of the bench surface, and then the area was sampled for aerosol contamination.

Summary of results

Multi-channel pipette

Sampling was performed starting with a decontaminated work area. Ten plates were inoculated and sealed, after which the area around the inoculum reservoir and plate were systematically sampled with contact plates for possible aerosol contamination. This was repeated three times, for a total of thirty inoculated plates; three separate sampling events for the *Serratia*, and thirteen events for a total of one hundred thirty plates for the Mycobacterium. After inoculation, ≥75% of samples collected near the seed tray reservoir containing the inoculum were positive. The remaining positive samples were collected within three or four inches of the plate. See the Sampling Grid; Figure 1.

The grid between the reservoir and plate yielded positive samples, but the frequency was less than the opposite side of the inoculum reservoir for the *S. marcescens* compared to the *M. fortuitum*, which showed about the same contamination on both sides of the reservoir (Figure 1). The testing of the two organisms was performed by two technologists, which shows there are slight aerosol pattern differences, however, results are consistent with pouring the inoculum into the seed tray.

Sensititre AIM Automated Inoculation Delivery System

Sampling was performed starting with a decontaminated work area. Ten plates were inoculated, removed and sealed, after which surfaces were systematically sampled with contact plates for possible aerosol contamination. This was repeated twelve to thirteen times for a total of one hundred twenty to one hundred thirty plates inoculated, and twelve to thirteen separate sampling events. The Sampling Grid in Figure 2 is a compilation of these sampling events for *M. fortuitum*. After inoculation, no positive samples were recovered from the sampling grid, with the exception of the immediate area where plates were placed during seal application, thus showing a lack of aerosol generation during inoculation, and that contamination, aside from the immediate surfaces of the AIM, results from plate manipulation after inoculation. Sampling from this area, in total, were too numerous to count (TNTC); however, single event sampling ranged from TNTC on one event to zero positive samples for other sampling events. There was, however, an incidence of positive samples surrounding the immediate area around the dosed plate on the Sensititre AIM surfaces. This included the pump assembly, directly under the plate, the plate holder, and on the cover surrounding the black surface. The incidence of positive samples from single sampling events ranged from >30 colonies on two sampling events to zero positive samples on four sampling events.

The plate holder and black instrument surfaces were flooded with isopropyl alcohol, wiped and allowed to air dry for a minimum of ten minutes at the end of testing. Sampling was repeated on all surfaces of the instrument from which positive samples had previously been recovered. No positive cultures were recovered, showing decontamination to be effective.

Plate surface sampling (*Serratia marcescens* only) prior to placing a seal on a plate

Sampling the plate surface prior to seal application resulted in positive samples for all cases. These sample colony counts were TNTC. Bubbles in the well can be formed during inoculation. Aerosols can be generated when a bubble bursts. The extent of generation of such aerosols is limited, with most ending up on the plate surface or on surfaces in the immediate proximity. The aerosol almost immediately dries on the plate surface and is contained when the plate seal is applied. This was also observed with the multi-channel pipette, but in this case, it is most likely due to the pipette tips touching the surface of the plate, or from missing the well as the inoculum is pipetted into the plate. For details of the performance of the Sensititre AIM System with Sensititre plates, refer to the Sensititre AIM User Manual.

After applying a seal on a plate

The incidence of positive samples from plates shown to have a significant number of positives on the plate surface was reduced to zero after a seal was applied. Placing a seal on the plate eliminates the risk of contamination from handling the plate after inoculation.

A further precautionary measure is to wipe the plate with an appropriate disinfectant. One hundred twenty-five plates were inoculated with the Sensititre AIM instrument and sealed. The surface was then wiped with isopropyl alcohol and allowed to dry. Each plate surface was sampled using contact plates. There were no positive samples for both the Sensititre AIM instrument and pipette.

Drop test sampling

Two plates were inoculated, sealed and dropped from twelve inches onto the solid surface. Plates were tested after inoculation and after incubation. No positive samples were recovered from the vicinity of the drop zone, thereby demonstrating that the seal adequately contains liquids when placed properly on the plate.

Conclusions and recommendations

The Sensititre AIM System or manual multi-channel pipette can be used by microbiology laboratories to inoculate plates for susceptibility and identification testing of bacterial isolates, following the technical insert and laboratory SOPs. This limited study showed that aerosol generation using either *Serratia marcescens* or *Mycobacterium fortuitum* gave similar results.

Areas of potentially greater aerosol generation have been identified for both procedures, and care must be taken to minimize the aerosol generation in the BSC. For example, if the multi-channel pipette is used, caution should be taken when pouring the inoculum into the reservoirs. Careful decontamination of the area in front of the inoculators after use should be performed. Careful seal placement is also important to contain the inocula in the wells of the plate after inoculation. If the plates are moved to another laboratory location, a secondary plastic bag to contain the plate(s) could be used. Laboratory procedures developed for handling hazardous (BSL-3) isolates, such as *Mycobacterium tuberculosis* complex, should always be followed.

The compact size of the new Sensititre AIM instrument allows technologists to easily place the unit inside a safety cabinet. The results from this experiment indicate less aerosol generation from the Sensititre AIM instrument, when compared to the multi-channel pipette, and much less contaminated disposables. However, both methods can be safely performed with trained personnel under a biological hood for BSL-3 isolates.

Recommendations

- Perform inoculation procedures within the confines of a biological safety cabinet for BSL-3 isolates.
- If using pipettes and open troughs, minimize aerosols by carefully pouring the inoculated broth into the reservoir.
- Discard the used dosehead and broth tube immediately after inoculation of the MYCOTB plate.
- Wipe the surface of inoculated plates with an appropriate disinfectant (such as Amphyl® or any phenol-based compound) before removing the plate from the safety cabinet.
- Wipe the surface of the Sensititre AIM instrument or pipette with disinfectant after each use.
- Use a secondary plastic bag if the plates are moved to another laboratory location.

Figure 1. Sample grid for the multi-channel pipette.

Average colony counts per ten plates for *Mycobacterium fortuitum* using a multi-channel pipette and reservoir for one hundred thirty plates.

G								
F			0	0 (1)	0			
E	0.04	0.11	0 (1)	0	0.6	0.2	0.1	
D	0	0.02	Plate	0.4 (1)*	Reservoir	0.6	0	
C	0	0.2 (1)*		0.3 (2)*		0.7	0	
B	0	0.1		0.7	2.1 (1)*	0.5	0.02	
A			0.4	0.6	0.4			
	4	5	6	7	8	9	10	11

*-( ) number of times TNTC was observed in that grid.

Figure 2. Sample grid for the Sensititre AIM instrument.

Average colony counts per ten plates for *Mycobacterium fortuitum* using a Sensititre AIM instrument for one hundred thirty plates.

G		0	Sensititre AIM Instrument					0	
F		0						0	
E		0						0	
D	0	0						0.02	0
C	0	0						0	0
B		0.02	0.08	0.02	1.5 (1)*	0.18	0	0	
A			0.02 (1)*	0.02	1.2 (1)*	0.2 (1)*	0	0 (1)*	
	4	5	6	7	8	9	10	11	12

*-( ) number of times TNTC was observed in that grid.

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