Contamination Control and Environmental Monitoring Program Results from a GMP QC Cell-Based (Bioassay) Lab; Risk Analysis and Recommendations

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PPD's QC bioassay laboratories in Middleton, Wisconsin, U.S., and Athlone, Ireland, previously reported observations regarding contamination control (CC) and environmental monitoring (EM) made over time and across program changes (*Pharmaceutical Outsourcing*, March/April 2016). In that article, the well-documented complexity and varied mechanisms of action (MOA) of biotherapeutic medicines were reviewed and discussed. Also included was a discussion about quality expectations established to ensure that potency testing required for the release of biotherapeutics is adequately performed (ICH Q6A) to determine product performance consistency across lots and time.

The application of standard cell culture techniques applied in support of *in vitro* potency testing and the risk of microbial contamination capable of altering the cell responses elicited (Miller, C.J. et al., Biotechniques. 35:812, 2003) with the potential to negatively impact test conclusions were reviewed. A conclusion was that QC bioassay laboratories should be and are generally designed, equipped and controlled to prevent culture contamination using effective CC and EM programs and analysts trained in both the application of the QC test methods and proper aseptic technique (Fresney, Basic Principles of Cell Culture; *Culture of Cells for Tissue Engineering*, Ch. 1 eds., John Wiley & Sons, Hoboken, NJ, 2006; and Phelan, Basic Techniques in Mammalian Cell Tissue Culture, Curr. Protoc. Cell Biol. 1.1.1 - 1.1.18, September 2007).

In this article, extended analyses of EM compiled contamination burden and rates were performed from the following available records: PPD's Middleton B5 cell-based assay laboratory for a period from the start of record keeping (March 2010); PPD's Middleton B8 cell laboratory suite, from the start of the GMP operations (May 2013); and PPD's Athlone cell bioassay laboratory suite from the start of the GMP operations (October 2014). Specific reference is made to additional EM performed during the period from October 1, 2015-October 31, 2016, that included increased gowning (use of disposable, whole body lab suits and boot covers); validation of disinfectant effectiveness and added sporocide application to biosafety cabinet (BSC) material decontamination; increased EM (monthly culture lab frequency and quarterly for support areas); and weekly biosafety cabinet (BSC) in-use viable contaminant monitoring. Also reviewed is the performance of the laboratories in the context of specific alert and action limits for EM contamination levels defined by PPD's cell laboratory SOPs, applied to aid in investigation and resolution of CC failure from identified EM trends.

The value of such measures, and in particular, BSC glove EM monitoring, is considered toward assessing the risk/benefit of such measures in reducing/preventing culture contamination that could delay or prevent effective assessment of biotherapeutic potency by bioassays validated for determination of product quality. The effectiveness of these CC and EM measures is reviewed relative to other measures PPD's QC bioassay labs have observed to be relevant toward controlling and monitoring these laboratories for microbial contamination.

PPD's GMP QC bioassay labs apply ISO 14644 (class 8), U.S. Food and Drug Administration (FDA) Sterile Drug Products Guidance (2004) and USP <1116> (controlled support), and Eudralex Volume 4 Annex 1 (grade D) manufacturing cleanroom recommendations/requirements. Intended for use in supporting aseptic manufacturing, they include expectations for inclusion of routine and 'frequent' environmental monitoring to detect contamination sources, with burden (conforming

to FDA and Eudralex guidance, Table 1) or frequency (conforming to USP <1116>, Table 2), and greatly exceed the cell and tissue culture processes generally applied to support and perform bioassays (Table 3).

Across PPD QC bioassay labs during the 13-month period reviewed here, sporadic viable contamination was widely observed (Table 4) and, in all but seven sporadic instances (<0.01% of EM events) across all bioassay labs and sites, the BSCs met EU grade B (equivalent to ISO class 5, FDA "aseptic processing" manufacturing) viable contamination targets.

Assessing the increased facility CC and/or EM results based upon USP <1116> contamination frequency guidelines, both sites culture labs' BSCs met controlled support manufacturing cleanroom contamination frequency limits across the respective periods (Table 5) in all measurements except for glove contamination and, only in the U.S. B8 culture lab, the observed viable air contamination frequency (10.25%) was slightly over the <10% limit across the 13-month period (compared to 3.7% reported after three months), but was markedly reduced in the Athlone culture lab (1.3%) compared to previously reported results (20.8%). A reduced frequency of observed contamination also was observed for settle plate contamination outside the BSCs for the Athlone cell culture lab (reduced from 20.8% to 4.5%). As in the previous report from multiple records, contact or swab EM results in Athlone demonstrated that cleaning procedures reduced contamination frequency, but did not eliminate it (Pharmaceutical Outsourcing, April/May 2016).

PPD's QC bioassay laboratories operate under defined limits for environmental non-viable particulate contamination alerts and action levels within and external to the BSCs used to perform cell culture and potency assays (Table 6a), as well as viable contamination within (Table 6b) and external to the BSCs (Table 6c). These are based upon regulatory expectations toward periodic EM sampling and a determination of contamination levels. The B5, B8 and Athlone QC bioassay lab EM results are reported annually or semi-annually (B5), and mycoplasma contamination from routine EM or of qualified cell banks has never been detected since implementation (January 2010). Reviewing all EM records for all QC bioassay labs through October 31, 2016, within and external to the BSCs,

Table 1. Regulation or Recommendations for Microbiological "In Operation" Cfu Limits

	EU Annex 1	04 FDA	USP <1116>
Aseptic Core	A <1	<1	<3
Aseptic Processing	B <10	n/s	<20
Controlled Processing	C <100	<10	<100
Controlled Support	D <200 air <100 settle <50 contact N/A glove	<100	n/s

Table 2. USP <1116> Recommended Contamination EM Frequency and Incidence Rate Limits

Grade	Frequency	Active Air sample	Settle Plate	Contact or Swab	Glove or Garment
Isolator (>ISO5)	Every shift	<0.1%	<0.1%	<0.1%	<0.1%
ISO5	Every shift	<1.0%	<1.0%	<1.0%	<1.0%
ISO6	Every shift	<3%	<3%	<3%	<3%
ISO7	Every shift	<5%	<5%	<5%	<5%
ISO8	2x/week	<10%	<10%	<10%	<10%
Less critical ISO 8 support areas	1x/week	<10%	<10%	<10%	<10%

Table 3.

PPD applied CC practices, compared with those described for basic aseptic cell culture principles (italics denote measures not described in basic principles):

- *Access is restricted*, the air supply is HEPA filtered and positively pressured to the external facility
- *Disinfectants have been validated for effectiveness in application on lab materials and surfaces*
- *All materials and equipment undergo disinfection prior to entry to the laboratory, using access controlled pass-through chambers that are regularly disinfected*
- *A cleaning and disinfection program is applied to the laboratory with a frequency determined to reduce or prevent the contamination*
- *An EM program regularly checks the level of contamination in the air, on lab and equipment surfaces, and within biosafety cabinets (BSC)*
- Hands are washed, *safety glasses are decontaminated, and disposable* lab coats, *hair and beard nets, and shoe covers are applied within a separated gowning room and sterile gloves* are *required for all BSC activities*
- All newly received cell lines are quarantined and handled with care *in a separate laboratory* until testing concludes the absence of mycoplasma, *even when purchased or received from sources providing sterility/mycoplasma free certification*

Table 4.

PPD B8 and Athlone Oct 2015-Oct 2016 (Culture Labs Only) Laboratory Contamination Load (high Cfu/site[a])

	Viable Air		Contact or Swab		
Location (EM frequency)	Post-clean	Settle	Pre-clean	Post-clean	Glove
B8 BSCs (all events)[b]	1	1	N/A[c]	5	137
Athlone BSCs (weekly events)[b]	1	1	1	1	16
B8 Culture Lab (monthly)	17	N/A	N/A	28[d]	N/A
Athlone Culture Lab (monthly)	14	3	34[e]	4	N/A
Athlone Assay Lab (monthly)	5	1	3	8	N/A
Athlone Other (quarterly)	42[f]	1	4	4	N/A

[a]Not all excursions are identified when multiple excursions of EM occurred of the same type
[b]BSC measurements (except viable air) were performed during/after use
[c]Not collected or not included in the analyses
[d]Post-clean floor contact
[e]Pre-clean floor contact
[f]Post-clean floor contact

Table 5.

PPD B8 Oct 2015-Oct 2016 (Culture Lab Only) and Athlone Jan-Jun 2015 and Laboratory Percent Contamination Frequency

	Viable Air		Contact or Swab		
Location (EM frequency)	Post-clean	Settle	Pre-clean	Post-clean	Glove
B8 BSCs (all events)*	<0.1%	0%	N/A	2.0%	11.9%
Athlone BSCs (weekly)*	2.9%	4.5%	N/A	1.6%	22.7%
B8 Culture Lab (monthly)	10.3%	N/A	N/A	3.4%	N/A
Athlone Culture Lab (monthly)	1.3%	4.5%	15.0%	8.6%	N/A
Athlone Assay Lab (monthly)	5	1	3	8	N/A

*BSC measurements (excepting viable air) were performed during/after use

Table 6a. Non-viable particulate sample contaminant alert and action limits for general lab areas and BSCs

Location	Alert Value	Action Value
General Cell Laboratory Areas	≥ 5,000 particles ≥0.5 µm	≥ 10,000 particles ≥0.5 µm
Biosafety Cabinets	≥ 5 particles ≥0.5 µm	≥ 10 particles ≥0.5 µm

Table 6b. BSC viable sample microbial contaminant alert and action limits

Location	Alert Value	Action Value
Air Samples, Per Site	≥ 1 cfu/ plate	≥ 2 cfu/ plate
Surface and Equipment Swab Samples, Per Site	≥ 2 cfu/plate	≥ 5 cfu/plate
Settle Sample, Per Site	≥ 1 cfu/ plate	Alert values observed on > 3 consecutive individual weekly EM samples
Glove Sample, Per Site	≥ 1 cfu/ sampling event (left and right plate combined is one sampling event)	Greater than 1 cell culture contamination event in the past 3 months per CL002 and alert values on > 50% of total weekly EM samples taken in the past 3 month time period (settle and glove)

Table 6c. Non-BSC sample site microbial contaminant alert and action limits

Location	Alert Value	Action Value
Air Samples and Bench Settle, Per Site	≥ 10 cfu/plate	≥ 16 cfu/plate
Surface Samples, Per Site	≥ 20 cfu/plate	≥ 25 cfu/plate

there have been no instances of non-viable air particle measurements at or above the alert level (Table 7). Viable contamination was observed sporadically by routine EM of air and surfaces (by both contact and swab and settle plates across all laboratories); and laboratory floors were the source of 62% of all alert and action limit events. Contamination alert and action events within the BSCs (set at much lower limits) represented about 29% of observations, and external surfaces outside the hoods were the location of 9%. Since the start of GMP operations in each of the laboratories, contaminations identified in this manner have never resulted in repeated alerts/action events with a frequency that would trigger an investigation.

As reported earlier (*Pharmaceutical Outsourcing*, March/April 2016), the EM program applied to PPD's QC bioassay labs tracks instances (including microorganism identification in the Middleton labs) of culture contamination events. Since record keeping was initiated (March 2012), only four culture contamination events occurred in PPD's B5 QC bioassay laboratory, all without an impact on QC testing or cell banking, and none have occurred since April 2013 (Note that CC and EM in this lab were not changed from the original programs). In the B8 cell laboratory, an increased contamination frequency was observed over a three-month period (July to September 2014). As reported, these did not correlate with an EM viable trend increase. However, an investigation was conducted that supported determination of root cause, with resulting corrective actions taken toward minimizing culture contamination across the labs. Since that report, only one other culture contamination was identified in PPD's B8 quarantine lab, during generation of a cell bank (the bank culture was terminated and repeated without incident). Genus and species of this contaminant (Bacillus altitudinis) was not associated with the aquatic environments related to the previously reported culture contaminants (various species of Brevundimonas, Burkholderia, Methylobacterium and Ralstonia). Since the start of operations in October 2014, the Athlone QC bioassay lab has had only one confirmed culture contamination event (organism identification was not performed).

It is also hypothesized that the validated system suitability specifications of bioassay methods used to evaluate biotechnology product potency should be sufficiently sensitive to changes that impact assay performance, including microbial contamination of cell cultures applied. Observations of increased and repetitive assay failures would be expected when CC measures were inadequate or improperly implemented. Such events

Table 7. Recorded EM sampling alert and action level events, PPD B5 and B8 Middleton, Wisconsin, and Athlone, Ireland, QC bioassay labs

		General Lab (#sites sampled)					
		Surfaces (62)		Floors (75)		BSCs (20)	
Sample Type	Lab ID (monitoring initiation date)	Alert	Action	Alert	Action	Alert	Action
Non-viable air particles	B5 lab (Jan-2010)	0	0	0	0	0	0
	B8 non-culture lab (May-2013)	0	0	0	0	0	0
	B8 culture lab (May-2013)	0	0	0	0	0	0
	Athlone lab (October-2014)	0	0	0	0	0	0
Viable air*	B5 lab (Jan-2010)	4	2	11	7	1	0
	B8 non-culture lab suite (May-2013)	1	0	15	3	0	1
	B8 culture lab (May-2013)	0	0	1	0	2	0
	Athlone lab suite (October-2014)	0	0	0	4	2	1
Viable Surface/Swab	B5 lab (Jan-2010)	0	0	1	2	0	0
	B8 non-culture lab (May-2013)	0	0	0	1	0	0
	B8 culture lab (May-2013)	0	0	0	1	1	1
	Athlone lab (October-2014)	0	0	0	1	6	0
Viable Settle	B5 lab (Jan-2010)	N/A	N/A	N/A	N/A	0	0
	B8 non-culture lab (May-2013)	N/A	N/A	N/A	N/A	N/A	N/A
	B8 culture lab (May-2013)	N/A	N/A	N/A	N/A	0	0
		0	0	N/A	N/A	7	0
	Total	5	2	28	19	19	3

*Not all general lab surfaces/floors had non-viable and viable air or settle plate sampling performed

should result in triggering GMP quality investigations that should enable identification of culture contamination as a root cause. Across PPD's GMP bioassay laboratories, it is estimated that more than 7,500 GMP QC sample tests have been performed. While it is possible that cell culture contamination has resulted in individual assay failures, few have been recorded as a demonstrated cause within PPD's QC bioassay labs and, more significantly, there has only been one inconclusive investigation initiated to determine whether an identified culture contamination might have compromised assay or analyst performance quality (associated with the only instance of culture contamination associated delay of a QC sample test, reported previously: *Pharmaceutical Outsourcing*, March/April 2016). The results of a passing sample that used cells prepared from the same culture later identified as contaminated were invalidated as a result of the possibility that they might have been impacted by coincidental contamination of the cells used for testing. The sample test was successfully repeated with a passing result.

As noted above, glove contamination monitoring was implemented and evaluated for analysts (n=31) working in the B8 cell culture lab BSCs, with EM sampling conducted after completion of cell culture activities. Results from 1,766 events were collected during the period from October 1, 2015 through October 31, 2016 and most events (1,556) showed no contamination. The observed contamination rate of 11.9% (Table 5) is similar to that previously reported for the first three months after glove contamination was monitored (9.6%, *Pharmaceutical Outsourcing*, March/April 2016). However, there appears to be a trend toward increased glove contamination (Figure 1) across the period since sample collection was initiated. Rates across analysts varied from 0-47%, and one analyst had one contamination event that exceeded the 100 cfu FDA Controlled Support cleanroom limit (Table 4). As noted above, regardless of the absence or presence of glove contamination, no contamination events were recorded within the B8 culture laboratory throughout this period (0 confirmed contaminations since September 2014).

Over this same period, glove contamination results also were tracked for analysts (n=10) in the Athlone cell culture laboratory, with 194 events recorded. As observed for the B8 culture lab, most EM showed no contamination, and individual analyst contamination rates varied widely (0-38%). Also like the B8 culture lab, a trend toward increased glove contamination rates was observed when comparing the rates of contamination originally reported in the Athlone lab from January-June 2015 (5.5%, *Pharmaceutical Outsourcing*, April/May 2016) and the October 2015-October 2016 results reported here (Table 5). The identified culture contamination event was not associated with positive EM results generated by that analyst on the date the culture was initiated.

The observed increase in glove contamination at both sites was the only trend identified across the EM performed.

Conclusions

The results reviewed here and previously have demonstrated that, throughout the service history of the PPD QC bioassay labs, application of validated CC measures (which includes analyst training in aseptic technique) and EM programs have demonstrated program efficacy roughly equivalent to aseptic manufacturing controlled support cleanroom expectations. These have been in place and, when appropriately applied, have demonstrated effectiveness in the generation of cell cultures and banks to support GMP-compliant product quality testing.

To address questions regarding the adequacy of the original PPD QC bioassay lab CC and EM programs, CC measures and EM were increased starting in October 2015. The accumulated results of added EM testing at two sites reported here, over an extended period (13 and 19 months, in the U.S. B8 and Athlone culture labs, respectively) failed to link BSC in-use contamination with culture contamination events. This confirms previous PPD bioassay B5 lab records accumulated over a multi-year period in which a slightly reduced BSC CC program and far more limited EM was applied with only infrequent culture contamination observed. Further, increased CC measures for either site did not appear to reduce observed glove contamination event frequency, relative to the original CC program results previously reported (*Pharmaceutical Outsourcing*, April/May 2016). The increased CC also had no apparent impact on culture contamination. However, throughout the period before and after that program change occurred, only two confirmed culture contamination events were observed across all three PPD QC bioassay laboratories over more than a two-year period, which makes drawing conclusions difficult. That this record has been observed despite the glove contamination frequencies observed clearly demonstrates that appropriate aseptic technique applied to cell culture is effective in controlling contamination, and should be emphasized within CC programs.

CC program effectiveness can also be monitored by a bioassay's or individual analyst's failure rate. However, using a conservative estimate of 7,500 GMP QC sample tests and 200 cell banks generated since March 2012, and with 22 recorded cell culture contaminations, there has been only one instance of a QC sample result determination being impacted (three-day testing delay) by cell contamination. It is unclear whether this is a reflection of the adequacy of the CC programs that have been in place, or whether other indicators such as contamination frequency and organism identification are more sensitive.

In PPD's experience, the most sensitive indicator of CC program effectiveness has not been the determination of contamination site load nor frequency, which are the expectations of current manufacturing cleanroom guidance. Rather, the most sensitive and specific indicator for identifying CC system ineffectiveness or non-compliance appears to have been recording culture contamination events. These have helped identify and establish contamination frequency and were instrumental in identifying patterns that indicated a lack of appropriate CC, and supported targeted improvements. Further, although it is unrealistic that conditions can be controlled to prevent all cell culture contamination, the subsequent records accumulated after improvements were implemented in October 2014 have demonstrated their durable effectiveness in reducing culture contamination events across PPD's GMP QC bioassay labs.

The reported data support a conclusion that PPD's original and correctly implemented CC program and improved training programs, when combined with reporting and assessment of culture contamination and assay failure events, appear adequate to ensure proper cleaning/disinfection is reducing contamination to a level that allows for the effective cell culture required to support GMP QC bioassay testing. These data also suggest that operations would not likely be compromised by a reduced EM plan similar to that originally in place for these laboratories.

The results demonstrate that GMP QC bioassay lab CC and EM programs can be effective without meeting the requirements of aseptic manufacturing core or processing cleanrooms. Expectations for generation and maintenance of a nearly aseptic environment in such facilities may be appropriate to prevent product contamination, but the added costs of such programs are not justified by the greatly reduced risk of contamination in a lab intended for testing product quality, with no direct risk to patient health.

In summary, in the absence of specific regulations, it is appropriate that QC bioassay labs follow available aseptic manufacturing-controlled support regulations and appropriate application of quality risk management, supported by facility performance histories, as directed by Eudralex Vol. 4 An. 1 (15). PPD's data suggest that an effective CC program that combines limited EM, monitoring of culture contamination frequency and organism identification, and includes GMP required fit-for-purpose application and monitoring of specific test method system suitability, can provide adequate control for such laboratories.