Extrapolating Testing for Biological Warfare Agents from the Laboratory to a Field Environment

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Field testing using live Biological Warfare Agents (BWA) has long been forbidden in the U.S. However, increasing BWA threats to both troops in the field and to the Homeland has made it imperative for the U.S. to develop systems that can detect and reliably identify BWA threats. Among the systems that the U.S. is developing is a "point" detection system that would be positioned in an area of potential BWA contamination to verify whether or not a BWA is present and to identify it if present. Testing of this system in a laboratory environment with live BWA is possible, but testing with live BWA cannot be done in the field. This paper describes a methodology to extrapolate results from laboratory testing through controlled open-air testing to a field environment. Both the overall methodology and the statistical methodology are discussed. Carefully parameterized logistic regression is the proposed statistical approach, and feasibility results based on previous field testing will be presented. Success of this methodology (if the proposed testing is executed) may be presented at a subsequent ACAS.

Introduction.

The four-service Joint Biological Point Detection System (JBPDS) is among the systems that the United States is developing to deal with the threat posed by Biological Warfare Agents (BWA). The JBPDS is an integrated system designed to automatically detect and identify the presence of BWA aerosols through direct contact with "clouds" potentially containing BWAs. Several versions of the JBPDS are available, including a relatively compact man-portable version that can be pre-positioned in an area potentially subject to BWA attack and a ground-mobile version that can rapidly be deployed to investigate suspicious clouds. The JBPDS provides an audible Alarm together with visual indication of the presence of BWAs displays their identification if any. It can also produce samples for transport to designated laboratories for confirmatory analysis.

As shown in Figure 1, the JBPDS is composed of four main line-replaceable units (LRUs): the Bio Agent Warning Sensor (BAWS), a wetted-wall cyclone collector/concentrator, the Fluidic Transfer System (FTS), and the identifier (Automated Hand-Held Assay, AHHA). An inlet duct on the BAWS LRU provides a pathway for sampling ambient air in close proximity to the instrument. The BAWS particle sensor constantly compares instantaneous measurements to an established background. A fluorescence detector, internal to the BAWS, determines if the sampled air contains aerosolized BWAs. If the BAWS alarms, the collector/concentrator collects and concentrates a sample that is passed by the FTS to the AHHA for identification of the BWA. When the AHHA receives the appropriate signal, a liquid sample from the FTS is automatically injected onto the assay strips. The strips, housed in a carrier, have identification markers that appear when a liquid sample of the BWA is inoculated onto the matching antibody strip. An optical reader of the carrier strips provides a means for identification.

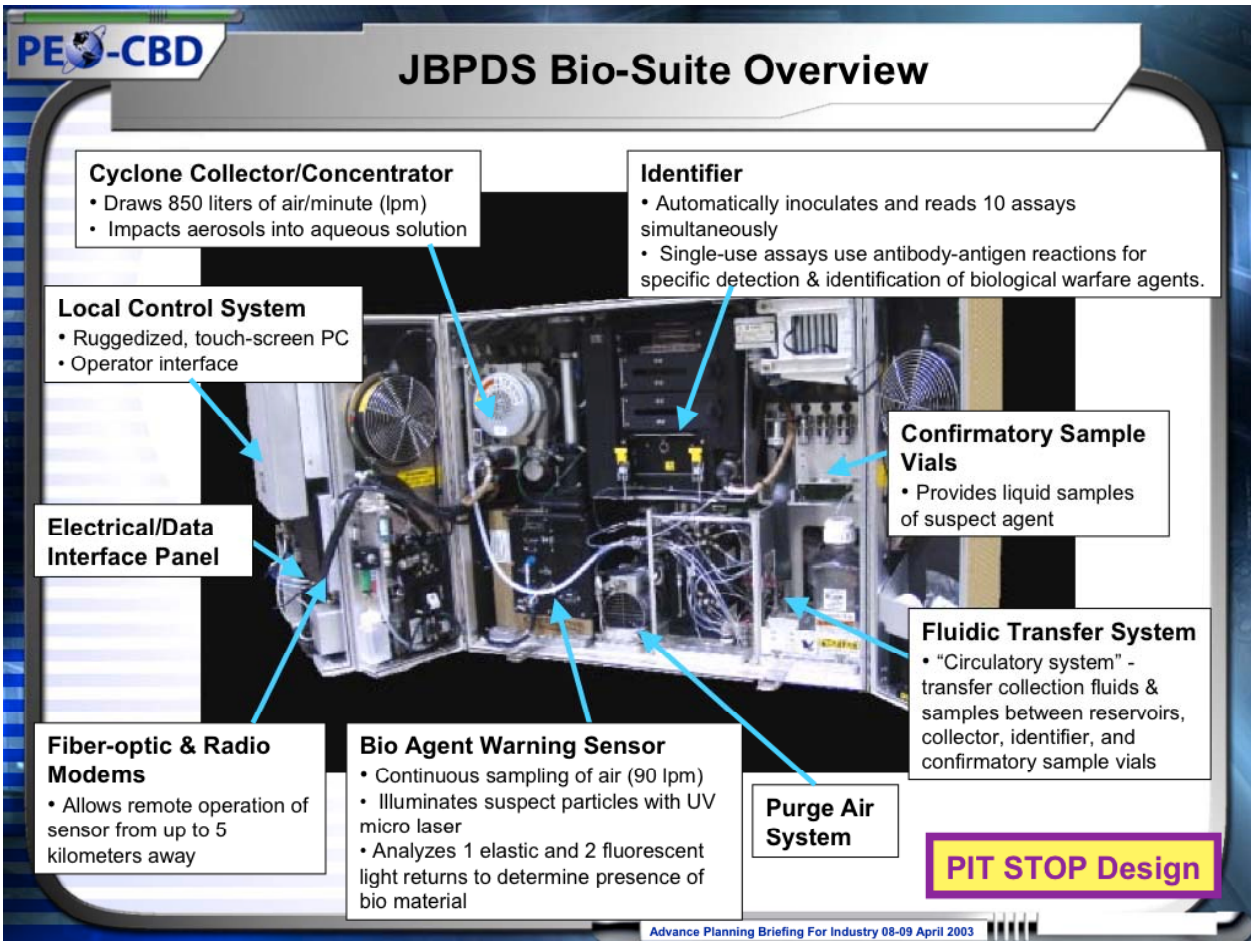

Figure 1. JBPDS Bio-Suite Overview.

The JBPDS has been tested in an enclosed containment chamber, in a controlled outdoor "Ambient Breeze Tunnel" (ABT), and in open field environments. Actual BWAs have only been used in the chamber, however, since outdoor testing with live/active BWAs (or even killed/inactivated BWAs) has long been forbidden in the U.S. In an open field environment (including the ABT), testing is only possible with killed/inactivated agent-like organisms (ALO). and/or live/active (or killed/inactive) biological simulants. However, no systematic study has yet been done to link performance of the JBPDS in the chamber with actual BWAs to performance of the JBPDS in outdoor environments with simulants and killed ALOs. In addition, it has not been possible to test the JBPDS as an integrated system in current containment chambers. There is also an issue concerning how the important cloud concentration factor is measured in the various test environments. Concentration is typically measured in terms of Agent Containing Particles per Liter of Air (ACPLA) for which there are several measurement methods, but the relationships between results for the various methods have not been thoroughly studied.

The Whole System Live Agent Test (WSLAT) is being proposed to address the issues outlined in the previous paragraph. First, WSLAT proposes constructing a containment chamber suitable for testing the smallest man-portable JBPDS configuration as an integrated system. Alternatively, disassembled JBPDS components would be tested simultaneously in existing chambers. For each of the four BWA agent classifications (spore bacteria, vegetative bacteria,

viruses, and toxins), WSLAT proposes to test both live/active and killed/inactive BWAs in the chamber along with both live/active and killed/inactive ALOs and live/active and killed/inactive simulants. Then killed/inactive ALOs and both live/active and killed/inactive simulants will be tested outdoors in the ABT and in open field environments. ACPLA measurement will be systematically addressed, but this paper will not cover the ACPLA issue in detail. In addition, the effects of particle size and cloud duration may be investigated.

Analysis Construct and Proof of Principle.

Although no systematic study has yet been done to link performance of the JBPDS in the chamber with actual BWAs to performance of the JBPDS in outdoor environments with live/active simulants and killed ALOs, limited test data are available to investigate whether such linkages are feasible. In particular, the JBPDS has undergone integrated system testing in the ABT and field with simulants, and the BAWS and the assay strips have been tested separately in a containment chamber with both live/active BWAs and live/active simulants. The following analysis construct and proof of principle exploits existing data to show that WSLAT is feasible from an analytic standpoint.

The existing test data for one agent classification were used to develop a logistic regression model for estimating JBPDS Prob[Alarm] and Prob[ID|Alarm] based on "Test" ("Chamber," "ABT," or "Field"), "Agent" ("BWA" or "Sim"), "Particle Size" ("Larger" or "Smaller"), and concentration (actually LogACPLA = Log_{10} ACPLA). The parameters obtained from that model were used to extrapolate chamber results for BWA to ABT and Field in a reasonable manner. Duration of exposure was not considered at this time due to insufficient data across tests. Particle size data was not available for ID data in the chamber, so particle size was not used as a fitting factor for Prob[ID|Alarm]. Examination of results by particle size for ID|Alarm data from ABT and field tests suggest that particle size has little if any influence on ID. Available particle size data relevant to Alarm were not very good, and they also had minimal effect; particle size was used in the model primarily to illustrate how such a factor might be incorporated.

Logistic regression is the standard statistical technique for modeling a discrete response variable as a function of continuous variables or a combination of continuous variables and discrete predictive factors. In the simplest case where there are only two values of the response variable (e.g., "Alarm" and "No Alarm" or "ID" and "No ID") logistic regression fits $\text{Prob[Alarm]}=e^{\mathbf{X}\boldsymbol{b}}/(1+e^{\mathbf{X}\boldsymbol{b}})$ (or $\text{Prob[ID|Alarm]}=e^{\mathbf{X}\boldsymbol{b}}/(1+e^{\mathbf{X}\boldsymbol{b}})$) where $\mathbf{X}$ is a matrix of coefficients and $\boldsymbol{b}$ is a vector of parameters. This is equivalent to fitting the log-odds ratio (ln{Prob[Alarm]/Prob[No Alarm]} or ln{Prob[ID|Alarm]/Prob[No ID|Alarm]}) as a linear model $\mathbf{X}\boldsymbol{b}$.

After much experimentation with available data, the following linear model was fitted using logistic regression to available JBPDS Alarm data for field trials and ABT trials and the chamber testing

$$\ln(\text{Prob[Alarm]/Prob[No Alarm]}) = \text{intercept} + t(\text{test}) + a(\text{test,agent}) + p(\text{particle size}) + c(\text{test,agent})*(\text{LogACPLA}-m) \quad (1)$$

where the shift parameter m is actually the overall mean of logACPLA. For simplicity, the intercept, test, and agent parameters for each model were grouped together to give an overall "group" parameter g(test,agent) given by

$$g(test,agent) = intercept + t(test) + a(test,agent) - c(test,agent)*m. \quad (2)$$

Then the reparameterized model was:

$$\ln(Prob[Alarm]/Prob[No\ Alarm]) = g(test,agent) + p(particle\ size) + c(test,agent)*LogACPLA. \quad (3)$$

Entertaining the notion that the ratio of the BWA slope for the ABT to the BWA slope for the Chamber should be the same as the ratio of the simulant slope for the ABT to the simulant slope for the Chamber (and similarily for the field) gives the constraints:

$$c(ABT,BWA)/c(Chamber,BWA) = c(ABT,Sim)/c(Chamber,Sim) \quad (4a)$$

and

$$c(Field,BWA)/c(Chamber,BWA) = c(Field,Sim)/c(Chamber,Sim) \quad (4b)$$

Extrapolating group parameters for ABT and field tests was not so straightforward, but a simple rationale enabled the desired extrapolation. Let c_{50}(Test,Agent) be the concentration at which Prob[Alarm]=0.5 (estimated from the logistic regression). A reasonable constraint for extrapolation of BWA chamber performance to the ABT is that

$$c_{50}(ABT,BWA) - c_{50}(Chamber,BWA) = c_{50}(ABT,Sim) - c_{50}(Chamber,Sim). \quad (5a)$$

Likewise, a reasonable constraint for extrapolation of BWA chamber performance to the field is that

$$c_{50}(Field,BWA) - c_{50}(Chamber,BWA) = c_{50}(Field,Sim) - c_{50}(Chamber,Sim). \quad (5b)$$

Since c_{50}(Test,Agent) occurs when Prob[Alarm]=Prob[No Alarm] (i.e., ln(Prob[Alarm]/Prob[No Alarm]) = 0), it follows from formula (3) that (ignoring the effect of particle size since it does not presently depend on test or agent)

$$c_{50}(Test,Agent) = -g(test,agent)/c(test,agent). \quad (6)$$

Applying constraints (5a) and (5b) together with formulas (4a), (4b), and (6) gives

$$g(ABT,BWA) = g(Chamber,BWA)*c(ABT,Sim)/c(Chamber,Sim) + g(ABT,Sim)*c(Chamber,BWA)/c(Chamber,Sim) - g(Chamber,Sim)*c(Chamber,BWA)*c(ABT,Sim)/\{c(Chamber,Sim)\}^2 \quad (7a)$$

and

$$g(Field,BWA) = g(Chamber,BWA)*c(Field,Sim)/c(Chamber,Sim) + g(Field,Sim)*c(Chamber,BWA)/c(Chamber,Sim) - g(Chamber,Sim)*c(Chamber,BWA)*c(Field,Sim)/\{c(Chamber,Sim)\}^2. \quad (7b)$$

The notion that slopes and relative positions of c_{50} for BWA potentially vary in accordance with equations (4a), (4b), (5a), and (5b) is speculative at this point. However, the charts in Figure 2 (derived by pasting logistic regression output from the SAS JMP statistical package into a Microsoft Excel workbook and performing the calculations described above) indicate reasonable-looking extrapolations from the available test data. For analysis, ACPLA measurements for Chamber testing were scaled similarily to ACPLA from ABT and Field testing. The extrapolations reflect not only the facts that c_{50}(Chamber,BWA) was about half of c_{50}(Chamber,Sim) and that the simulant curves for ABT and field tests were shifted to the right from the chamber test but also the flattening of curves for ABT and Field tests.

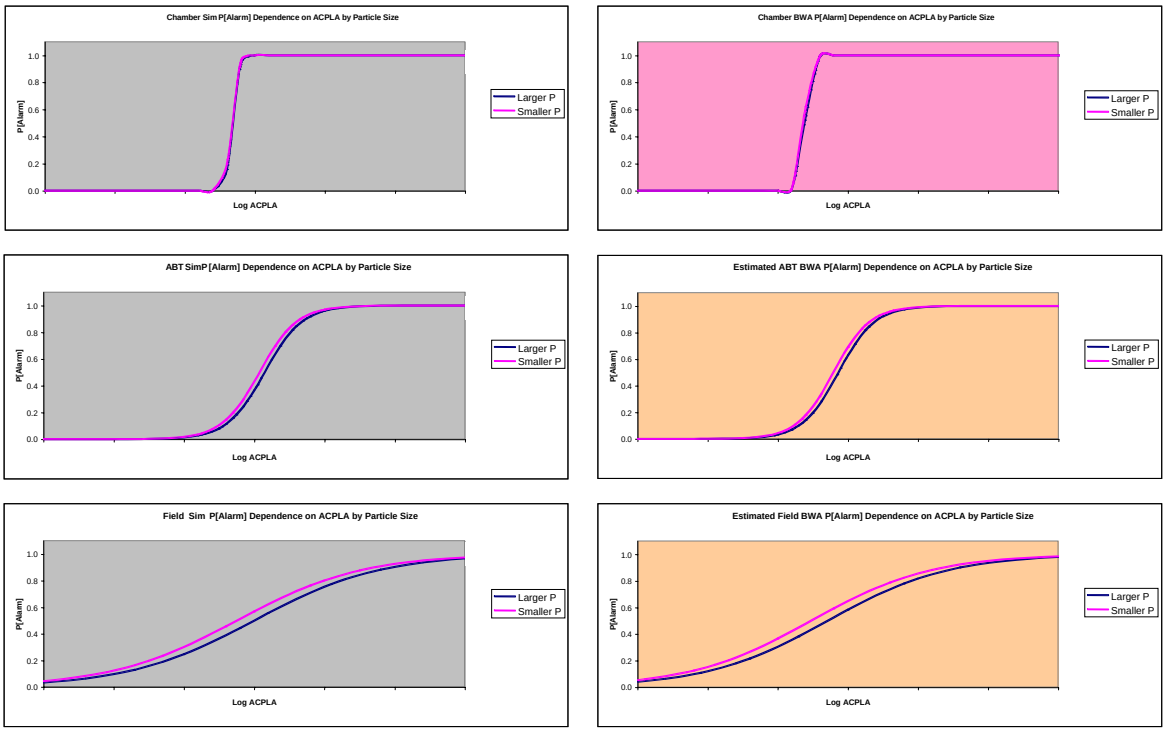

Figure 2. P[Alarm] Dependence on ACPLA by Test Environment and Particle Size
(Grey Background Indicates Simulant, Pink Background Indicates BWA, Orange Background Indicates Extrapolated BWA)

Treatment of ID|Alarm data was very similar to that for the Alarm data. The following linear model (identical to formula (1) for Alarm except that no attempt was made to fit particle size since no particle size data were available for the chamber)

$$\ln(\text{Prob[ID|Alarm]}/\text{Prob[No ID|Alarm]}) = \text{intercept} + t(\text{test}) + a(\text{test,agent}) + c(\text{test,agent})*(\text{LogACPLA}-m) \quad (8)$$

where as before, the shift parameter m is actually the overall mean of LogACPLA. As with Alarm data, the intercept, test, and agent parameters for each model were grouped together to give an overall "group" parameter g(test,agent) given by

$$g(\text{test,agent}) = \text{intercept} + t(\text{test}) + a(\text{test,agent}) - c(\text{test,agent})*m. \quad (9)$$

Then the reparameterized model was:

$$\ln(\text{Prob[ID|Alarm]}/\text{Prob[No ID|Alarm]}) = g(\text{test,agent}) + c(\text{test,agent})*\text{LogACPLA}. \quad (10)$$

Logistic regression output was again pasted into a Microsoft Excel workbook and equations (4a), (4b), (7a), and (7b) were used to calculate revised and extrapolated parameters. As with Alarm data, the charts from Excel displayed in Figure 3 show that the extrapolations give reasonable results. In chamber ID|Alarm testing, solutions of known concentration were inoculated directly onto the identification media, so that ACPLA was known very precisely and the curve inflections are very sharp. If there had been more uncertainty in chamber ACPLA determination, curves for chamber ID|Alarm data would have been much flatter (as they were in ABT and field tests where ACPLA was determined with more uncertainty). The extrapolations

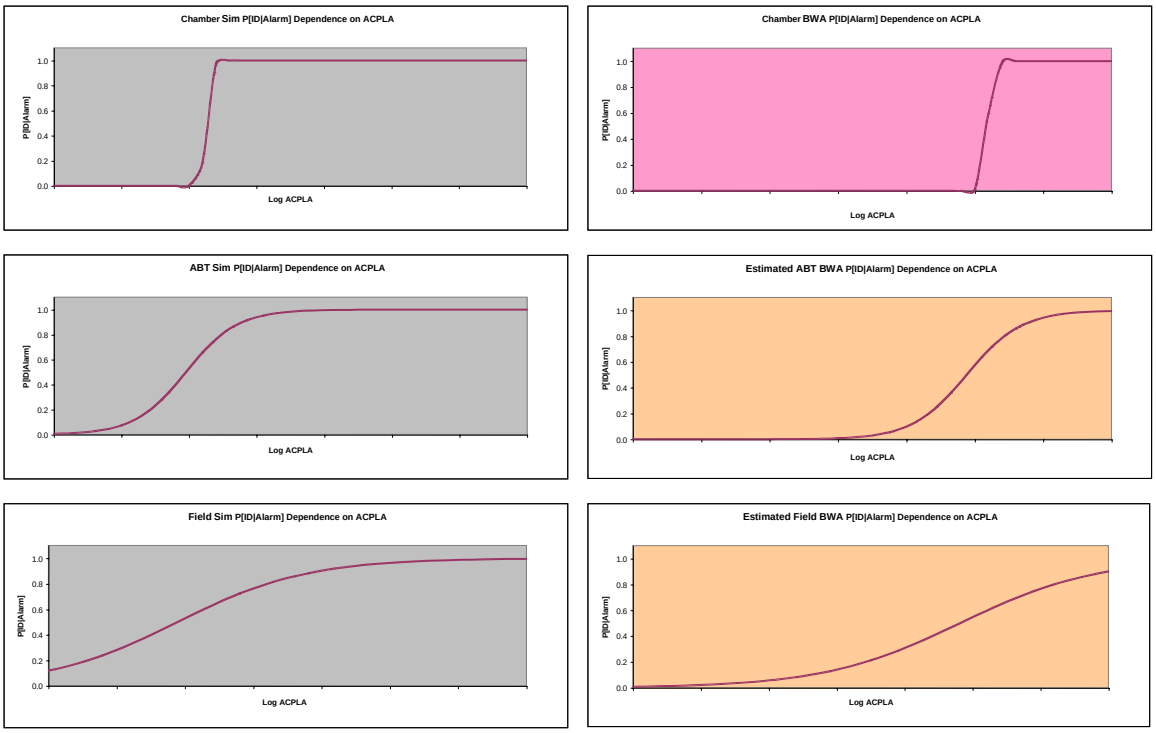

Figure 3. P[ID|Alarm] Dependence on ACPLA by Test Environment
(Grey Background Indicates Simulant, Pink Background Indicates BWA, Orange Background Indicates Extrapolated BWA)

used reasonably reflect flattening in ABT and field tests by shrinking the c(Chamber, agent) parameters. As expected, they also capture the slight shift to the left suggested by simulant data for the ABT and field tests.

Extension to Full WSLAT.

If WSLAT is conducted, it is anticipated that the analysis construct used above will be the starting point for analysis. In addition to possibly having more than one particle size for each Agent, “Shorter” and “Longer” durations are possible for cloud releases, and there will be a “Live/Killed” status factor. In particular, it is anticipated that both live/active and killed/inactive BWAs as well as both live/active and killed/inactive ALOs and simulants will be used in each agent classification (spore bacteria, vegetative bacteria, virus, and toxins). The live/active and killed/inactive BWAs will be released only in WSLAT chambers, but it is anticipated that both live/active and killed/inactive ALOs and simulants will be released in the ABT and the field. This will provide the ability to crosswalk the transformations used to extrapolate current BWA chamber data to ABT and field environments between live/active and killed/inactive simulants, between live/active simulants and killed/inactive ALOs, etc. Examination of these relationships in WSLAT test data may build confidence in the ratio approach or suggest a better approach. It is anticipated that separate fits would be done for each agent classification. Tentative factors and levels for WSLAT are listed in the following table.

Factor	Parameter	Nesting	Levels	Level Labels
Test	t	None	Chamber, ABT, Field	C,A,F
Particle Size	p	None	Larger, Smaller	LP,SP
Duration	d	None	Larger, Smaller	LD,SD
Agent	a	Test	BWA, Sim	B,S
Status	s	Test, Agent	Live/Active, Killed/Inactive	L,K
Concentration	c	Test, Agent	NA (coefficient)	

The initial log-linear model to be entertained for Alarm is:

$$\ln(\text{Prob[Alarm]}/\text{Prob[No Alarm]}) = \text{intercept} + t(\text{test}) + p(\text{particle size}) + d(\text{duration}) + a(\text{test,agent}) + s(\text{test,agent}) + c(\text{test,agent})*(\text{LogACPLA-m}) \quad (11)$$

and a similar model will be entertained for ID|Alarm. Reparameterization and extrapolation is expected to proceed as it did for currently available data in this analysis construct.