

High Performance xMAP assay using AssayCheX™ Process Control Panel

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Abstract

To enhance overall performance of xMAP based multiplex assay system we developed the AssayCheX™ Process Control Panel. Inclusion of this panel in multiplex xMAP immunoassays will significantly improve the quality of the detection system by monitoring the proper conduct of an assay. The inclusion of the process control panel assures the validity of results. These control microspheres monitor the error introduced by instrument or operator failure during the performance of an assay. The usefulness of the AssayCheX process control panel in a multiplexed immunoassay system was demonstrated for simultaneous quantitation of eleven different analytes using xMAP technology. All analytes were tested in a sandwich immunoassay format. Capture antibodies were coupled to microspheres and detection was done using a mixture of different biotinylated antibodies. Streptavidin-phycoerythrin was used as fluorescent reporter. This study compares the quality of data obtained in the multiplex assay with and without the inclusion of AssayCheX process control panel. We did not see any interference in quantitation of unknown analytes by including the process control panel. When samples are run in replicates the operator or instrument error may increase the coefficient of variance. Customized post acquisition data analysis software was used for flagging of invalid results. Removing the flagged values from final analysis of test analytes improves the quality of data by reducing the variance amongst the replicates.

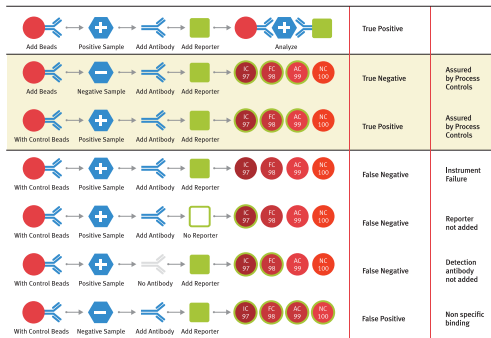
Introduction

Use of multiplexed technologies is being preferred as they provide high content information. Luminex® xMAP® technology has become a method of choice as one can simultaneously analyze multiple targets within one reaction or one sample. We developed a custom xMAP immunoassay for quantification of eleven analytes in one sample. This 11-plex suspension array gives a higher dynamic range and better limit of detection when compared to ELISA. We used selected detection and capture antibodies to develop sandwich immunoassay on microspheres for different analytes. First a single plex assay for each analyte was developed and then all the analytes were multiplexed to obtain the resultant suspension array.

This poster shows the benefits of including AssayCheX process control panel in a custom array. The AssayCheX process control panel was designed to provide reassurance that the results are valid in every well / test sample of any immunoassay using Luminex xMAP technology. Use of this panel in every well enables a user to disregard or fail any individual well that may have invalid data due to operator or instrument error. The Four-plex microsphere panel in AssayCheX utilizes two independent acceptance metrics to ensure that each step of the immunoassay is performed correctly and that the instrumentation is operating properly at the time of data acquisition. Inclusion of these controls in every well enables a user to invalidate an entire plate in the event of an instrument failure or a kit failure or to ignore individual sample data in the event of an intermittent instrument error or sporadic operator error. Figure 1 displays how the AssayCheX™ panel monitors different steps of an immunoassay.

Figure 1

Immunoassay Scheme with or without AssayCheX™ Process Control Panel



Materials and Methods

A custom multiplexed suspension array for quantitation of eleven different analytes developed in house was used for this study. AssayCheX™ PCP 01 lot # V081215CM01 from Radix BioSolutions was used.

Assay parameters: 11-plex capture antibody microspheres were mixed together with AssayCheX PCP 01 to give a 15-plex microsphere mixture. Each sample was tested in triplicate. The same samples were tested both in presence and absence of AssayCheX 4-plex panel. The assay was done in 96 well filter membrane bottomed microtiter plates (Multiscreen Vacuum Manifold, Millipore). The plate wells were wetted using assay buffer before starting the assay. 50 µL of samples was added to each well to which 50 µL of 11-plex / 15-plex mix was added and capture reaction was allowed to incubate at room temperature for one hour. Two washes were performed using a vacuum device with 100 µL of wash buffer. Next incubation was done with biotinylated detection antibody mixture for one hour at room temperature. After incubation and 2 washes, the fluorescent reporter Streptavidin-Phycoerythrin conjugate was added and incubated as described above for 30 minutes. The plates were washed 2 times and microspheres resuspended in 100 µL of assay buffer and analyzed using BioPlex System and BioPlex Manager software Version 4.1 (BioRad, Hercules, CA).

Data Analysis: The data acquired using BioPlex manager was exported as an excel file and analysed using MasterPlex QT 4.0 software with AssayCheX Module from Hitachi Software Engineering America, Ltd. for data analysis. MasterPlex QT software analyzes results files (*.csv, *.xls, *.lxd or *.mix*) from the Luminex® 100/200 or BioPlex system.

Figure 2A
Overlay of the titration curves for 11 analytes without AssayCheX addition

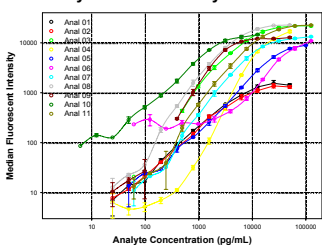


Figure 2B
Overlay of the titration curves for 11 analytes with AssayCheX addition

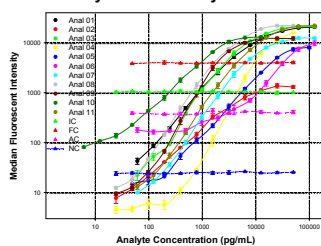


Table 1
Percentage recovery two sample controls for 11 different analytes

Name	Percentage Recovery			
	With AssayCheX		Without AssayCheX	
	Known Sample 1	Known Sample 2	Known Sample 1	Known Sample 2
Analyte 01	86.7	105.5	105.7	77.9
Analyte 02	80.5	104.3	82.4	79.0
Analyte 03	96.8	109.3	89.5	77.1
Analyte 04	91.2	105.4	93.2	98.4
Analyte 05	82.6	83.2	70.3	65.0
Analyte 06	104.7	93.0	72.6	181.9
Analyte 07	81.6	103.9	107.2	85.2
Analyte 08	87.2	102.8	92.9	83.0
Analyte 09	81.3	101.5	184.3	93.5
Analyte 10	85.1	101.2	105.0	77.3
Analyte 11	88.9	105.0	91.5	81.2

Results and Discussion

The eleven standard curves obtained without and with AssayCheX added are shown in figure 2A and 2B respectively. It is apparent that standard deviations in values without AssayCheX are much higher. Those wells were not flagged and hence used for analysis. The flagged wells were reviewed for errors and data was reanalyzed when AssayCheX were used.

Table 1 shows that recovery of the concentration of the known samples is not affected by addition of AssayCheX. There were 3 values that did not meet acceptable recovery range of 70%-130%. Since no AssayCheX was used there it could not be assigned to any instrument or operator error.

Figure 3
Screen shots from AssayCheX module of MasterPlex QT 4.0

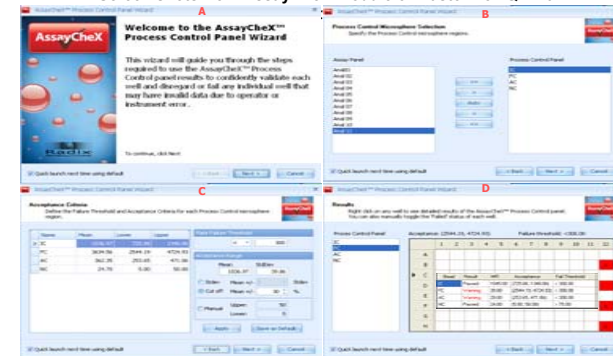


Figure 3 shows the screen shots of the MasterPlex QT 4.0 software with AssayCheX Module from Hitachi Software Engineering America, Ltd. Figure 3A shows the opening menu of the AssayCheX module. Figure 3B shows the display of the selection of process control panel in the module. We use a simple algorithm as described below for flagging the results that may have some error. Figure 3D indicates there were 4 wells that were flagged. They show that fluorescence reporter was not added to those wells. That shows error on both microsphere region 98 and 99.

This flagging algorithm uses two acceptance metrics. The first acceptance metric is composed of a set of threshold values for the AssayCheX™ panel. These threshold values are designed to function as an entire plate diagnostic procedure to determine any error due to catastrophic instrument or reagent failure. Such an error can result in false negative values that are overlooked in the absence of AssayCheX™. In the software window they are called "Plate Failure Threshold" (Fig. 3C).

The second acceptance metric consists of a range of acceptable fluorescence values for each AssayCheX™ microsphere set to ensure that each well of an assay plate has been processed properly and that the instrument has operated properly at the time of data acquisition. Acceptance criteria values are presented as a range of high and low acceptance values to accommodate normal variance. In the software window it is called "Acceptance Range" (Fig. 3C). Sample results in which the AssayCheX™ results fall within the acceptance range are considered valid. Whereas, sample results in which the AssayCheX™ results are outside the acceptance range are identified as potential invalid results. (Fig. 3D). The pattern of AssayCheX™ values may also be used as a troubleshooting guide to indicate which specific process of the assay has failed.

Summary

Multiplexed xMAP immunoassays have a clear advantage over the conventional ELISA. One can detect and quantify multiple analytes simultaneously in a large number of samples. Each microsphere is equivalent to one assay and each signal is a median of 100 reactions per sample. One limitation here is that in absence of proper controls one is not always confident about the negative results. Failure of analysis instrument or operator error can cause false negative results. Also when one is testing multiple samples some matrices may be exceptionally sticky and cause non specific binding resulting in false positives. If automation is used for addition of reagents the robotic arm error could also cause false negatives.

Integration of the AssayCheX™ process control panel helps one gain confidence in complete process of performance of assay and hence data quality. The AssayCheX algorithms developed for analysis of data using software that flags the wells that may have caused an error due to failed instrument or reagent helps one eliminate those specific data points and not retest the whole sample or plate. The Process Control Panel can also be integrated seamlessly with various commercial multiplex immunoassay kits.