

ALIS-IC 2000 QUANTITATIVE MODULE & ITS USE IN FACILITATING STUDIES UTILIZING MULTIPLE BIOMARKERS

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Introduction

Biomarkers are indicators of a biological response related to disease progression or remission. Carefully selected biomarkers can be useful in the selection of a lead compound, determining the mechanism of action of a compound, be used as a surrogate endpoint for demonstrating efficacy, or for identifying intermediate endpoints of success to decrease follow-up time with a specific treatment. Many biomarkers are in an ELISA format and can be either in the form of a kit or custom assay. The validation of these immunoassays according to GLP, final sample analysis with a panel of biomarkers, and the monitoring of assay performance requires the processing of various forms of data and multiple data points per patient or specimen.

Biomarker assays that are used in drug development and in preclinical and clinical studies may be custom assays or commercially available kits. In either case, the assay needs to be put through a full method validation of (1) Precision and accuracy (spike recovery levels) (2) Sensitivity and specificity (3) Recovery (may expect over recovery due to endogenous levels) (4) Linearity, parallelism, and curve fitting (5) Matrix effects, and (6) Stability studies. In addition to these validation runs, the in-study runs need to be continuously monitored for performance by tracking the reproducibility of the standard curve, QCs and normal, as well as, baseline samples. When considering that many biomarker studies include many more than a single assay, the task of data collection and analysis can be quite

difficult. Each subject will have multiple time points, with each time point being analyzed on as many as 6-10 different assays – some in plasma, some in serum. This requires an efficient data management system specifically designed to manage and track information such as analytical method revision control, sample tracking, plate preparation and analytical results for multiple biomarkers in multiple matrices and allowing production of client specific reporting with ease. FDA has recently published guidance¹⁻⁴ that address issues pertaining to computerized systems used to create, modify, maintain, archive, retrieve, or transmit clinical data intended for submission to FDA and provide Guidance and criteria under which FDA will consider electronic records equivalent to paper records and electronic signature equivalent to traditional handwritten signature.

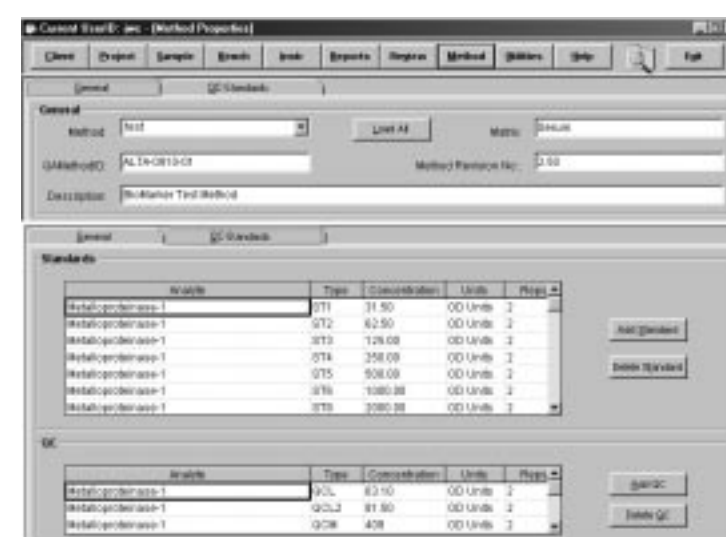
ALIS-IC 2000 software incorporates features such as encrypted electronic signatures, complete audit trail and password protected task based access that allows us to meet several GLP compliance requirements for automated systems and satisfies the needs of these demanding studies. The use of ALIS-IC 2000 allows bidirectional data transfer. A sample sequence run list is generated and uploaded to StatLIA for instrument control and the results of assay are transferred back to ALIS-IC 2000 using an automated instrument upload module. The assay results are then processed based on parameters defined by study, method and protocol. Finally the analytical results are reported using ALIS-IC 2000's Seagate Crystal Reports or via a custom designed MS Word 97/2000 VBA application⁵.

1 The Immunoassay Work Flow - Sample Login, Plate Assignment, Sample Run List Generation and Assay Data File Upload

A typical phase II clinical study may include measurement of multiple biomarkers (8 to 10 biomarkers), some of which are in an ELISA format. Some of the assays are validated in plasma and others in serum, depending on the analyte and what matrix would be appropriate for a reliable measurement of disease response. Patients would be recruited over approximately a 6-9 month period and each blood draw (both plasma and serum would be drawn at the designated time point) would be divided into 200 uL aliquots, some being serum and some being plasma. (This is to eliminate any issues associated with freeze thaw or refrigerator storage.) Each subject's aliquots would be given a unique Lab. Sample ID upon accessioning and relevant data would be collected from the tube label. Each time point aliquot (plasma or serum) would be assigned to the appropriate biomarker assay by selecting the sample with the appropriate matrix and entering that time point sample into the work list for that full plate assay. The next time point for that subject, again in the appropriate matrix, would be selected for addition to the same run list and plate setup. This would repeat until all of that subject's time points were included on this one specific biomarker assay. A different biomarker would need for the sequence to repeat (with new aliquots) from the beginning time point and continue until all time points were again included on this second biomarker.

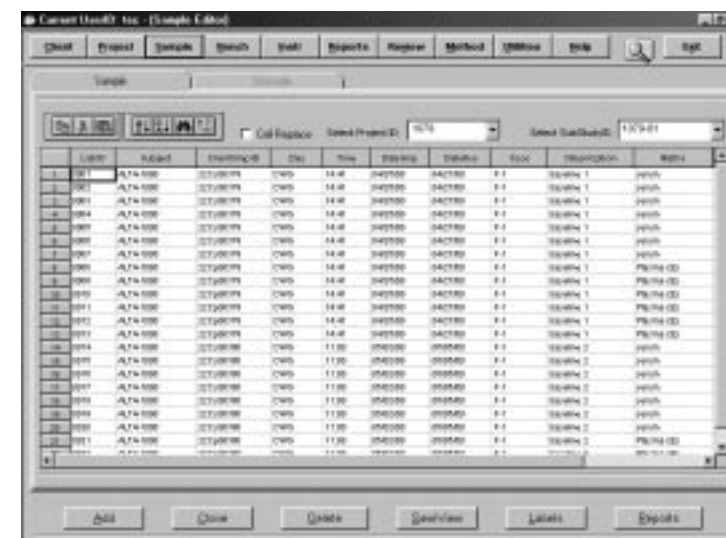
Immunoassay run is initiated by production of a sample run list file containing Plate Sequence Position, Lab. Sample ID, QC Code and Batch Number by ALIS-IC 2000. The StatLIA software reads this sample sequence run list and acquires the data. Upon completion of the run, the data is processed and exported back to ALIS-IC 2000 system. The ALIS-IC 2000 system is designed to generate a sample sequence run list file in StatLIA's "LIMIN" file directory and the exported files are transmitted directly to "ALIS-IC\ProjectData" directory, thereby minimizing any manual intervention. ALIS-IC 2000 system is designed to read a single or multiple assay export files interactively or in a batch mode and user can interactively access data in a multigrid display screen - assay data and associated statistics.

Analytical Method Setup



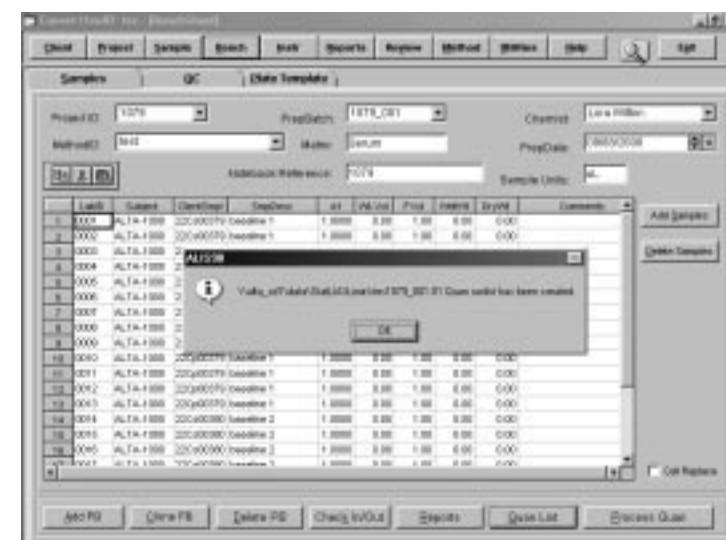
- Analytical Method Name and Number
- Analyte and QC Levels.
- Reports

Sample Login



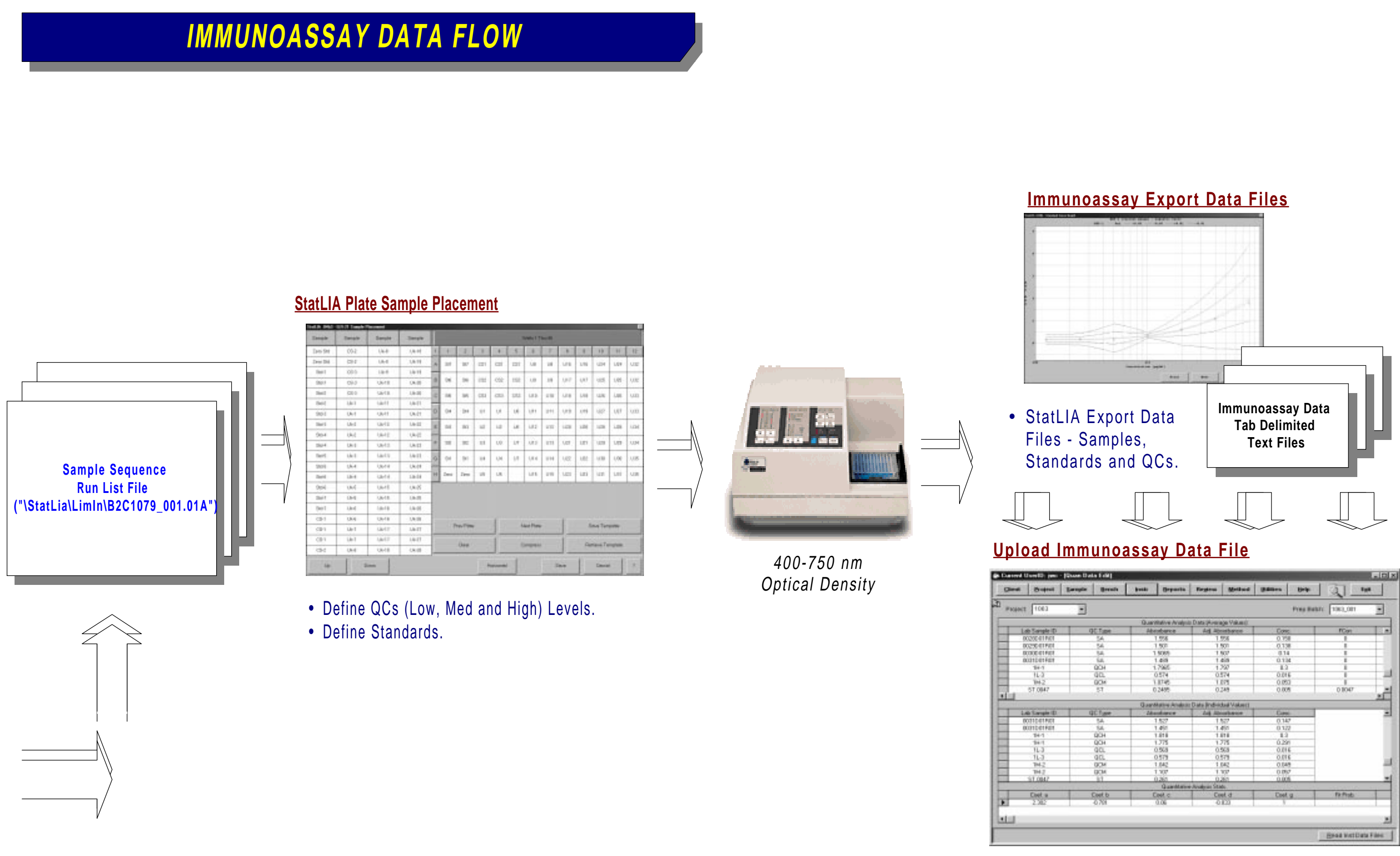
- Lab Sample ID, Client Sample ID, Subject ID, Day, Time, Matrix, Date Sampled, Date Rec'd, Sample Description, Storage Location, etc.

Sample Sequence Run List



- Prep. Batch No., Matrix, Analytical Method, etc.
- Define Sample Replicates and Dilutions.
- Run List Generation.

IMMUNOASSAY DATA FLOW



2 Data Reporting Using MS Word-97/2000 VBA Automation & Customized ALIS-IC 2000 Datasheets

A custom Visual Basic for Applications (VBA) program⁵ is utilized to extract processed data from ALIS-IC 2000 stored in Access97 database tables. The module then populates predefined Word-97/2000 report templates using the extracted data. The user interface containing all quantitative report components is displayed and the user can interactively select one or all tables, example table outputs are shown below. Quantitative reports are automatically numbered, paginated and stored in a designated project directory. The use of VBA/Word 97/2000 based templates allows for a rapid customization based on client requirements and inclusion of these final report output tables in a variety of customer defined file formats such as EXCEL, WORD, etc. In addition, custom ALIS-IC 2000 datasheets gives point and click access to assay results for unknown samples, control samples (QCs) and standards using a drag and drop Segate Crystal Report Writer.

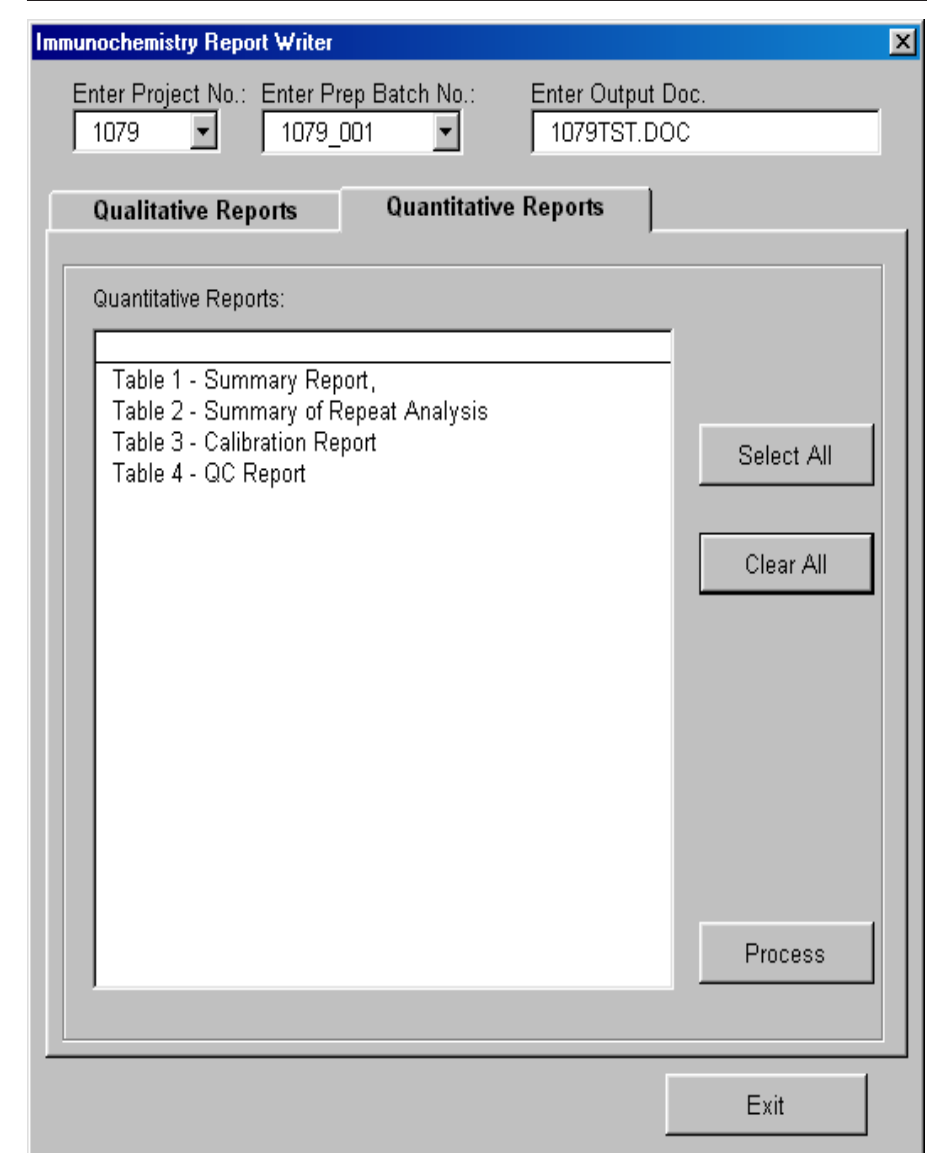


Table 1. Multiple Biomarker Summary Report

SUBJECT ID	SAMPLE DESCRIPTION	DRAW DATE	DRAW TIME	Human Matrix Metalloproteinase-9		Vascular Endothelial Growth Factor		Cross-Linked N-Telopeptides		Human Tissue Inhibitor of Metalloproteinase-1		BOI PHOSPHA Assay Batch N
				Assay Batch No.	Results (ng/mL)	Assay Batch No.	Results (pg/mL)	Assay Batch No.	Results (nM BCE)	Assay Batch No.	Results (ng/mL)	
SBJ-01	SAMPLE DESC: 1234	09/06/2000	09:00	1063_001	67.217	1063_002	117.371	1063_003	16.75	1063_010	24.11	1063_0
SBJ-02	SAMPLE DESC: 1234	09/06/2000	10:30	1063_004	72.411	1063_005	106.392	1063_006	21.00	1063_012	38.04	1063_0
SBJ-03	SAMPLE DESC: 1234	09/06/2000	11:45	1063_007	81.262	1063_008	125.763	1063_009	12.70	1063_015	47.08	1063_0

Table 3 Summary of Calibration Curve (Five Parameter Asymmetrical Logistic) Data for DRUG in Human EDTA Plasma

Assay Date	Assay Number	200 pg/mL Mean Conc. (pg/mL) (n=2)	100 pg/mL Mean Conc. (pg/mL) (n=2)	500 pg/mL Mean Conc. (pg/mL) (n=2)	250 pg/mL Mean Conc. (pg/mL) (n=2)	125 pg/mL Mean Conc. (pg/mL) (n=2)	62.5 pg/mL Mean Conc. (pg/mL) (n=2)	31.25 pg/mL Mean Conc. (pg/mL) (n=2)							
2/29/00	CYN-1	2001	0.1	1007	0.7	478	-4.4	267	6.8	133	6.4	70	12.0	10	-68
3/1/00	CYN-2	2002	0.1	1003	0.3	490	-2.0	252	0.8	147	17.6	64	2.4	15	-52
3/1/00	CYN-3	2001	0.1	996	-0.4	498	-0.4	252	0.8	133	6.4	63	0.8	28	-10

Table 4 Summary of Quality Control Sample Analyses for DRUG in Human EDTA Plasma

Assay Date	Assay Number	QC Low (100 pg/mL)			QC Med (600 pg/mL)			QC High (1000 pg/mL)		
		Mean Conc. (pg/mL) (n=3)	Precision (%CV)	% Accuracy	Mean Conc. (pg/mL) (n=3)	Precision (%CV)	% Accuracy	Mean Conc. (pg/mL) (n=3)	Precision (%CV)	% Accuracy
2/29/00	CYN-1	241	9.1%	120.5	660	1.5%	110.0	1037	3.3%	103.3
3/1/00	CYN-2	232	11.2%	116.0	702	5.6%	117.0	1074	7.6%	107.4
3/1/00	CYN-3	254	17.5%	127.0	752	3.4%	125.3	1188	5.0%	118.8

3 Conclusion

In summary, we have described our approach to automating the data management and reporting of multiple biomarkers in multiple matrices for quantitative analysis and integration of ALIS-IC 2000 with StatLIA data acquisition system. The use of sample sequence run list generation and completely automated instrument upload file modules helps eliminate transcription errors and increases productivity. ALIS-IC 2000's open architecture gives access to a multitude of reporting options - VBA based Word 97/2000 based templates and Segate Crystal Reports, thereby allowing us to customize and produce reports in a wide variety of formats - EXCEL, TXT, HTML, etc. based on client's specific requirements. Future work will focus on development of specialized biomarker data queries and custom reporting module designed around client specific requirements allowing access to immunoassay data via web in a secure (SSL enabled) intranet environment.

1. Electronic Records; Electronic Signatures: Final Rule 3/20/1997 FR Vol 62, No. 54, 13430-13466.
2. Guidance For Industry - Computerized Systems Used in Clinical Trials. FDA April 1999.
3. The FDA Inspection Program. J. McCormack. SQA Workshop Adv. QA Techniques, Alexandria, VA, July 1998.

4. Computers in Clinical Trials Draft Guidance. D. A. Lepay. FDA - Division of Scientific Investigation June 24, 1997.
5. Method Validation Report Generation Using Word97 Automation. John Cornacchia, Scott Serl, Jerri Willoh, Robert Bethem and Ike Tabani. 10th International Symposium on Pharmaceutical and Biomedical Analysis, Washington, DC, May 1999.