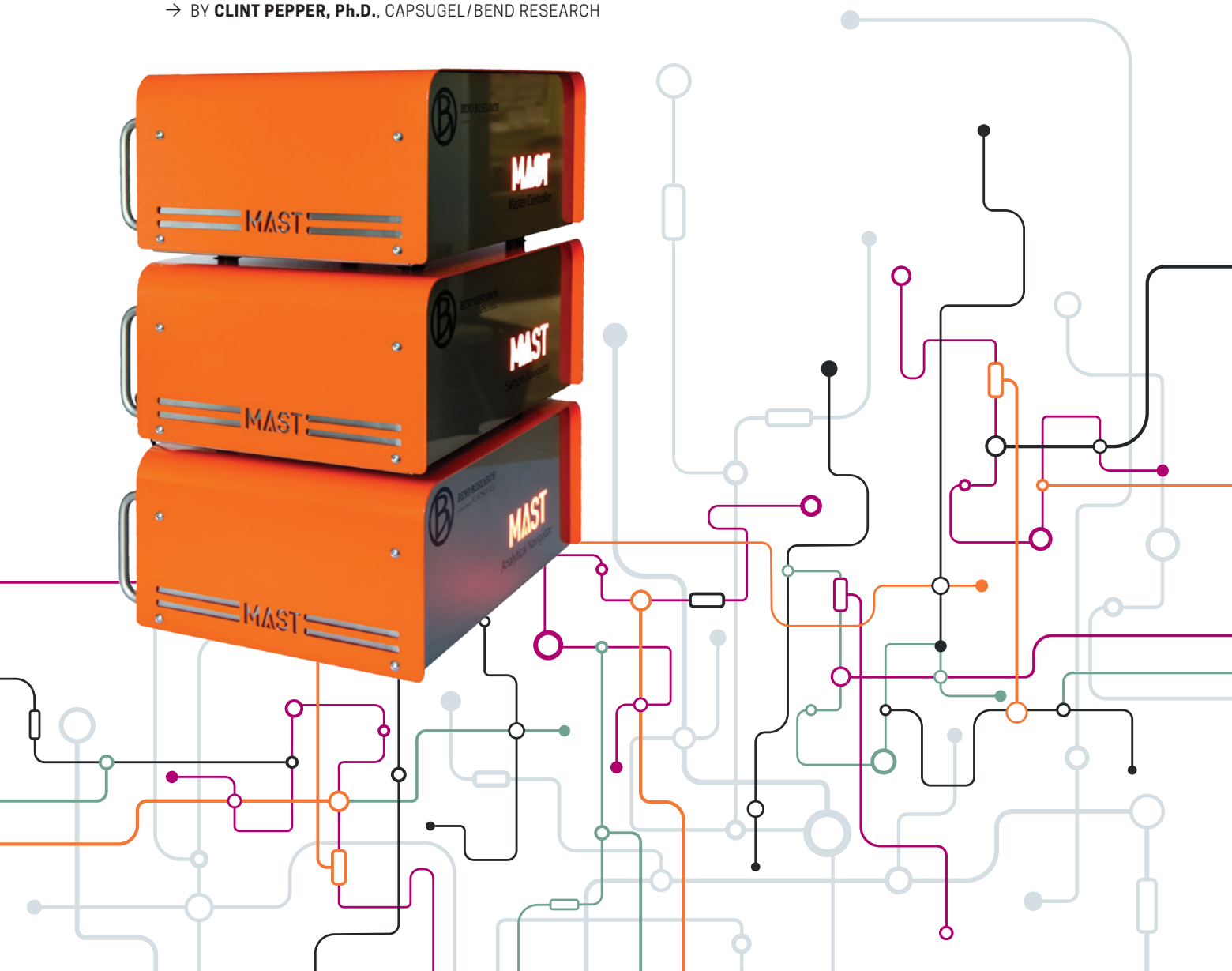
MAKING REAL-TIME PROCESS ANALYTICAL TECHNOLOGY IN BIOMANUFACTURING A REALITY

→ BY **CLINT PEPPER, Ph.D.**, CAPSUGEL/BEND RESEARCH



Automated, aseptic sampling and analysis is a prerequisite for making real-time Process Analytical Technology (PAT) in biomanufacturing a commercial reality. Developed in collaboration with several leading pharmaceutical companies over an extensive development program, the Modular Automated Sampling Technology (MAST) platform from Capsugel/Bend Research allows direct transfer of aseptically collected bioreactor samples to analytical devices, providing rapid, reliable data for superior bioprocess guidance.

WHY AUTOMATED SAMPLING SYSTEMS?

The FDA first introduced the idea of Process Analytical Technology (PAT) in its 2002 Vision for the 21st Century. It followed up with publication of a guidance document in 2004.[1] The agency defines PAT as "a system for designing, analyzing and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of better understanding processes and thus ensuring final product quality."[2] PAT is also essential to the successful implementation of continuous processing; real-time data is required for continuous control, which enables optimum operation during the entire run.

In the biopharmaceutical industry, real-time product quality attribute control is desired to maximize protein production and quality in bioreactors. Current noninvasive spectroscopic methods such as Raman, near infrared, and dielectric spectroscopy provide real-time information on cell culture and fermentation processes but are not able to product quality information.

A mechanism for obtaining real-time information through analyses that require sampling of the bioreactor (or sampling during downstream unit operations) is a prerequisite to gain better insight and understanding of bioprocesses, whether they are run in batch or continuous (perfusion) mode. To fully integrate PAT into bioprocesses and facilitate the evolution of the sector toward real-time data collection, product quality attribute control and overall bioprocess guidance, a reliable system is required to transfer bioprocess samples directly from bioreactors to analytical devices while maintaining process sterility.

MAST™: DEVELOPED FOR THE BIOPHARMA INDUSTRY

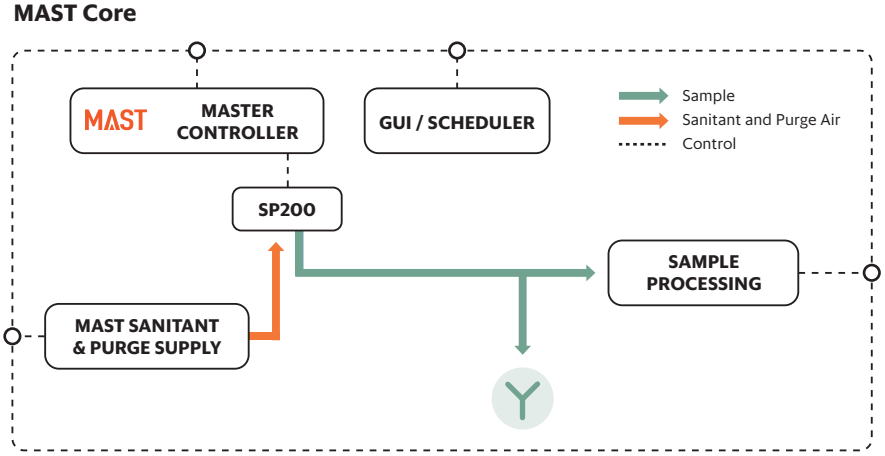
The Modular Automated Sampling Technology (MAST™) platform from Capsugel/Bend Research is a complete system specifically designed to fit this need and therefore facilitate improved bioreactor quality and yield. This first-of-its-kind technology allows aseptic collection of representative samples that generate detailed process information in real time. MAST is the result of an intensive five-year development program with pilot programs and significant input from major biopharmaceutical players, and a range of modules have been developed to provide customized sampling, interface and reporting.

Designed for use in both development and commercial-scale applications, MAST enables the collection of media, cell and product quality data across scales to provide:

- hands-off, contamination-free sampling;
- automated sample scheduling;
- automated at-line analysis of whole broth and cell-free samples;
- increased sampling frequencies;
- increased sampling reproducibility;
- increased data reliability; and
- redirection of saved resources to higher-value activities.

Automatated sample collection eliminates operator involvement, reducing the risk of contamination and operator exposure. The ability to collect more reproducible samples more frequently and integrate data from multiple analytical methods can accelerate process development. The data can also be used to develop more accurate predictive control models, which can in

FIGURE 1



THE MAST PLATFORM ALLOWS COLLECTION OF SAMPLES FROM UP TO 10 STERILE SAMPLE SOURCES AND **CAN DISTRIBUTE THOSE SAMPLES TO FOUR ANALYTICAL DEVICES FOR AUTOMATED ANALYSIS.**

turn enable the implementation of novel process control and product quality attribute control strategies. Furthermore, the MAST system allows operators to respond rapidly to changes in process conditions to maintain optimum bioreactor performance and maximize yields.

MAST: CUSTOMIZED TO EVERY APPLICATION

The MAST platform allows collection of samples from up to 10 sterile sample sources and can distribute those samples to four analytical devices for automated analysis. Due to its modular nature, the MAST platform can be tailored to the specific needs of each customer and bioprocess. One of the most important modules in the platform is the Sample Pilot™, which is designed to appropriate scale for development through commercial applications.

The Sample Pilot SP100 module is designed specifically for fixed stainless steel bioreactor applications. It can be used at the development to manufacturing scale and takes sample in 55 mL increments. The SP100 is constructed of PEEK (polyether ether ketone), a robust organic polymer thermoplastic known for its thermal stability. The sampling module is autoclave-sterilized and affixed to the bioreactor prior to the regular bioreactor Steam In Place (SIP) cycle. The sampling module is mounted to the bioreactor using an industry-standard 25 mm Ingold port and requires only a three-inch radius of space.

The Sample Pilot SP200 is designed specifically for development scale or single-use bioreactor applications. It can be used at the development to manufacturing scale and takes sample in 5 mL increments. The module is compact, requiring little space on a bioreactor (~2-inch radius). Installation is straightforward with multiple port connection options that allow integration directly into a single-use bioreactor bag using a Kleenpak connector sleeve, or insertion through a dip tube into a bench top development bioreactor. The SP200 can be used on bioreactors of all scales and can be adapted to all ports and fitting types.

Sanitation is designed into Sample Pilot operation. After each sample is taken, all sample contact components, including the Sample Pilot, are flushed with liquid sanitant and placed in a user-defined sanitant hold time. Once the hold is complete, the Sample Pilot and the associated sample lines are blown dry with compressed purge gas. Single-use purge gas and sanitant supply filter assemblies ensure that there is a consistent flow of these fluids from run to run.

The MAST platform includes software systems developed to monitor operation, manage scheduling, review historical data and manage system setting. The modules also feature an easy-to-use graphical interface.

CASE STUDIES

The following case studies provide specific examples of how the MAST platform is used to improve the performance of bioreactors and solve common industry problems.

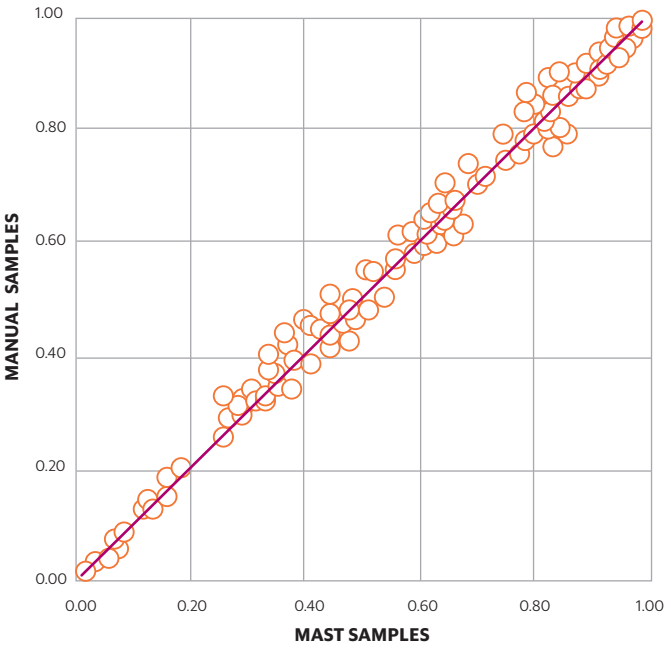
CASE STUDY 1: AUTOMATING SAMPLING AND ANALYSIS

The MAST platform was integrated with a Nova BioProfile FLEX automated sample analysis system, a unit widely used in biotechnology laboratories to determine viable cell density; metabolite, salt and dissolved gas concentrations; pH; etc. Results obtained via automated sampling with the MAST platform were then compared to those obtained with manual sampling.

In operation, the BioProfile FLEX system is integrated with the MAST system. Once the MAST platform confirms that the Nova system is ready, a sample is drawn according to the test parameters that have been entered into the MAST interface and sent to the MAST sample collection cell. The Nova sample probe then moves into position and draws the sample from the cell. After the testing is completed, the MAST system flushes the sample contact lines with sanitant and then blows the system dry with purge gas.

The MAST platform has been integrated

CASE STUDY 1



Total cell density, viable cell density and viability normalized parity plot **comparing MAST to manual samples**

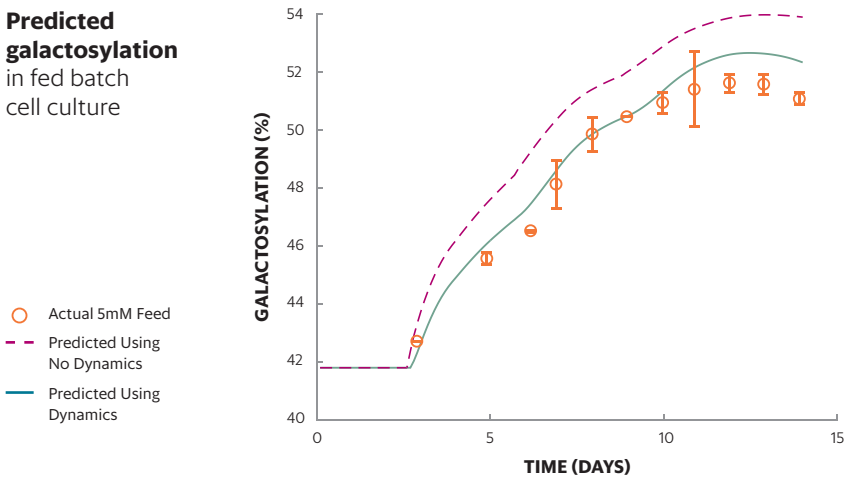
Normalized parity plot comparing MAST and manual samples measuring total cell density, viable cell density and viability from five different cell culture batches.

to multiple different Nova BioProfile FLEX units, and thousands of MAST samples have been automatically analyzed. For this study, results were analyzed from five cell culture batch runs (ranging from 1 to 500 liters) at end user and Capsugel facilities using Sample Pilot units (SP100 and SP200). Parity plots showed that the results for MAST samples automatically analyzed by the Nova BioProfile FLEX correlated well with the results for samples collected and processed manually.

It was concluded, therefore, that MAST system samples are consistent with manual samples and representative of the conditions inside the bioreactor. By enabling autosampling for routine assays, the MAST platform has freed up operator and testing resources otherwise necessary for manual testing.

CASE STUDY 2

Predicted galactosylation in fed batch cell culture



CASE STUDY 2: ACCELERATING DEVELOPMENT

The MAST system enables the collection of time series data from bioreactors at a sufficient frequency to capture dynamic behavior of cell culture processes. The dynamic data can then be used to develop dynamic models required for model predictive controllers. In this study, a predictive model was developed for the galactosylation of a monoclonal antibody expressed from a CHO cell line, based on different quantities of galactose in the feed.

A perfusion process with constant viable cell density, feed rate and volume was used as a steady-state reaction cell, and responses to changes in input variables were monitored. Once the antibody galactosylation reached a steady-state value, the culture was subjected to a step increase in galactose concentration and was allowed to reach a new steady-state antibody galactosylation. The galactosylation of the antibody product in the bioreactor was continuously monitored using a MAST platform at four-hour intervals. Each reactor had two SP200 Sample Pilots: one to draw whole broth samples, which were sent to a Nova BioProfile Flex instrument, and the other to draw cell-free samples from the permeate side of the perfusion system, which were sent to a Gilson liquid handler for analysis of percent galactosylation by high-performance liquid chromatography (HPLC).

The dynamic MAST data revealed that the cell response to the increase in galactose concentration was not instantaneous; rather, a time delay of ~12 hours was observed. A predictive model without this dynamic information overpredicted the galactose concentration with a steadily accumulating error, whereas the predictive model taking into account the dynamic data provided by the MAST system was more accurate.

CASE STUDY 3: ENABLING PRODUCT QUALITY ATTRIBUTE CONTROL (PQAC)

Automated sampling coupled with automated analysis of critical product quality attributes (PQAs) has been shown to enable implementation of PQAC schemes in bioreactor systems. Measurement of PQAs is a significant technical hurdle. PQAs may include glycosylation profiles, degree of aggregation, degree of amidation. etc. Techniques such as HPLC, Ultraperformance liquid chromatography (UPLC) or liquid chromatography/mass spectroscopy (LCMS) are often used.

After the sample is collected, it must be processed before it is injected into the analytical instrument. At a minimum, the cells must be removed before injection or the instrument can be damaged. The MAST Cell Removal System™ (CRS) uses tangential flow filtration technology to effectively isolate the cells from the whole broth in a retentate sample and collect cell-free permeate for transfer to downstream analytical devices. Up to 30 samples can be processed per CRS filtration cassette. The MAST system was designed to be configurable and flexible for easy cleaning and quick change out of filtration cassettes.

Further processing of the sample can be required after cell removal, including Protein A purification, solid phase extraction, dilution and digestion. Once processing steps are completed, the sample is transferred to the analytical device and automatically tested using a preselected method.

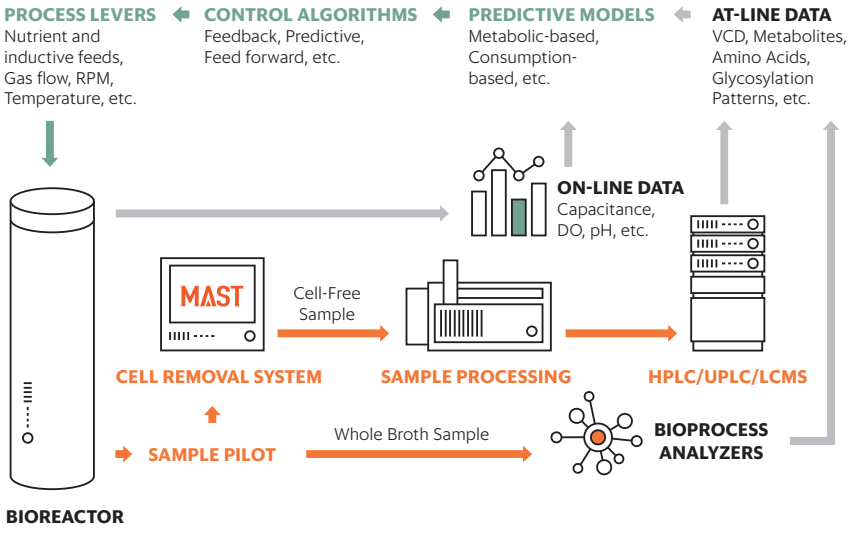
In this application, Capsugel has focused on Protein A purification and analysis using a Waters Patrol UPLC. After cell removal by the MAST CRS, the cell-free permeate is transferred to a Gilson liquid handler, where the samples are purified using an automated Protein A method. The accuracy and precision of the automated method were verified by comparing the results for six replicate samples purified using the automated method to those processed using a traditional GE AKTA Explorer method. Both methods yielded results within a standard deviation.

IN THE BIOPHARMACEUTICAL INDUSTRY, REAL-TIME PRODUCT QUALITY ATTRIBUTE CONTROL IS DESIRED **TO MAXIMIZE PROTEIN PRODUCTION AND QUALITY IN BIOREACTORS.**

CASE STUDY 3

MAST ACCOMMODATES TAILORED SYSTEM DESIGN AND **CAN BE READILY EXPANDED WITH ADDITIONAL SAMPLING MODULES AS REQUIRED.**

Process flow diagram for a potential **PQAC system**



The purified cell-free sample is then transferred to the Waters UPLC for analysis. MASTconnect™ software retrieves available Waters methods and makes them available during the sample scheduling process. When a sample is taken, the MAST platform communicates critical information (e.g., Sample ID, Experiment ID and sample start time) to the Waters system, ensuring sample traceability and data integrity. MAST monitors the progress of the UPLC, provides updates on progress and can send an alarm if issues arise.

The MAST platform controls all of the Sample Pilots, the CRS, the Gilson liquid handler and the solution supply systems, as well as communicating with analytical devices and other features through a series of modular control enclosures. MAST accommodates tailored system design and can be readily expanded with additional sampling modules as required. MASTconnect software allows configurable, flexible and user-friendly operation of the MAST system, with special modules for sample scheduling, sample navigation and analytical data management.

MAST: NOW COMMERCIALLY AVAILABLE AFTER RIGOROUS TESTING AND COLLABORATIVE WORK

The commercial availability of the MAST technology is the culmination of a focused five-year program conducted at Capsugel's Bend Research facility in Bend, Oregon, in collaboration with several of the world's largest biopharmaceutical companies. Capsugel has also developed alliances with numerous leading analytical equipment suppliers to facilitate PAT integration into bioprocessing with the MAST platform. These collaborations have enabled an optimized, automated sampling system to be developed that allows direct transfer of aseptically collected bioreactor samples to analytical devices, providing rapid, reliable data for superior bioprocess guidance.

Testing has been extensive, and has included high cell density cell culture bioreactors, viscous microbial applications and downstream sample collections. MAST systems have pulled thousands of representative samples from development scale bioreactors to 500 liter stainless steel bioreactors to 2,000 liter single-use bioreactors while maintaining the sterility of all samples.

Our rigorous testing and collaborative work with equipment manufacturers and end users has demonstrated the MAST platform's reliability, accuracy and value. MAST's integrated design has enabled increased sampling frequency and reproducibility, as well as improved data reliability when compared with manual sampling, and enables the bioprocessing industry to take a step forward in bioreactor control and yield. P

ABOUT THE AUTHOR

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Clint Pepper has spent more than 20 years in the biologics, pharmaceutical and medical device industries creating products, developing processes and manufacturing biopharm compounds in development, clinical and commercial environments. He has seen several products through from phase I to commercial approval. Clint currently helps Capsugel create the Modular Automated Sampling Technology (MAST) auto-sampling solution that can be used in any application where maintaining sterility of the manufacturing process is the highest priority.

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