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(54) Title: SYSTEMS AND METHODS FOR PREPARING A SAMPLE AND PERFORMING A REAL-TIME ASSAY OF THE SAMPLE

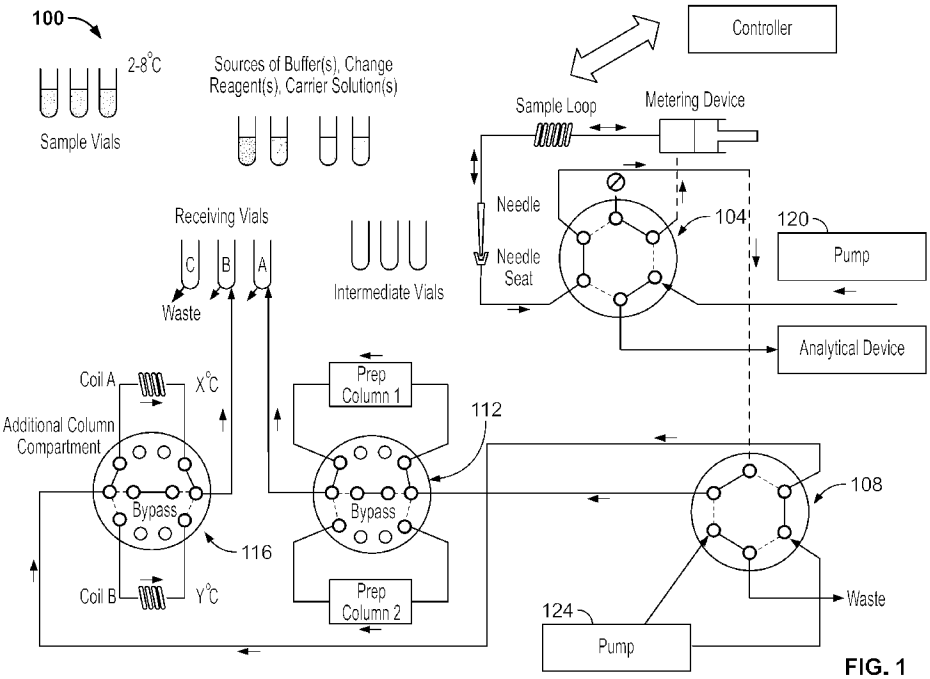


FIG. 1

(57) Abrégé/Abstract:
Systems and methods that facilitate the automatic (or substantially automatic) preparation of a sample of a product containing molecules for analysis and automatic (or substantially automatic) performance of an assay of that sample. Thus, the preparation and analysis can be performed substantially in-real time, or, in other words, much more quickly than presently allowed by conventional systems and methods.

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(54) Title: SYSTEMS AND METHODS FOR PREPARING A SAMPLE AND PERFORMING A REAL-TIME ASSAY OF THE SAMPLE

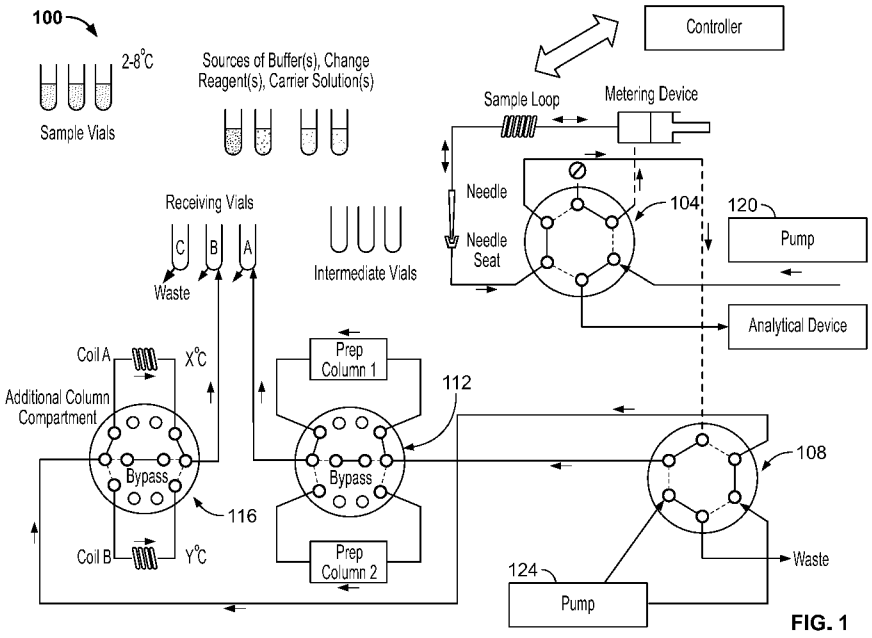


FIG. 1

(57) Abstract: Systems and methods that facilitate the automatic (or substantially automatic) preparation of a sample of a product containing molecules for analysis and automatic (or substantially automatic) performance of an assay of that sample. Thus, the preparation and analysis can be performed substantially in-real time, or, in other words, much more quickly than presently allowed by conventional systems and methods.

[Continued on next page]

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SYSTEMS AND METHODS FOR PREPARING A SAMPLE AND PERFORMING A REAL-TIME ASSAY OF THE SAMPLE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] Priority is claimed to U.S. Provisional Patent Application No. 62/805,902, filed February 12, 2019, and U.S. Provisional Patent Application No. 62/951,346, filed December 20, 2019, the entire contents of which are hereby incorporated herein by reference.

FIELD OF THE DISCLOSURE

[0002] The present disclosure relates generally to assays and, more specifically, to systems and methods for preparing a sample and performing a real-time assay of the sample.

SEQUENCE LISTING

[0003] The present application is being filed with a sequence listing in electronic format. The sequence listing provided as a file titled, "53661_Seqlisting.txt" created February 11, 2020 and is 263,959 bytes in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

BACKGROUND

[0004] Assays are commonly performed to quantify one or more attributes of an analyte such as a drug, a biochemical substance, or a cell. An example of such an assay is the multi-attribute method (MAM) assay, which can detect and quantify Critical Quality Attributes (CQAs), identified by the Quality Target Product Profile (QTPP), of a sample (Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics. Rogers RS, Nightlinger NS, Livingston B, Campbell P, Bailey R, Balland A. *MAbs*. 2015; 7(5): 881-90). The MAM assay is a manually-operated process that is performed in, for example, a Large Molecule Release Testing (LMRT) laboratory. MAM is a liquid chromatography (LC) – mass spectrometry (MS) -based peptide mapping method, having three steps: (1) sample preparation (which can include, for example, polypeptide denaturation, reduction, alkylation, and digestion); (2) separating the digested polypeptides by LC and detecting them by MS; and (3) analysis of the data for targeted CQAs and detection of new signal (*i.e.*, peaks) when compared to a reference standard.

[0005] CQAs are chemical, physical, or biological properties that are present within a specific value or range values. For example, for large polypeptide therapeutic molecules, physical attributes and modifications of amino acids (the building blocks of polypeptides) are important CQAs that are monitored during and after manufacturing, as well as during drug development. Unlike conventional analytical assays that track changes in peak size and peak shape of whole or partial polypeptides, MAM detects specific CQAs at the amino acid level.

[0006] Analysis of the glycan profile of a polypeptide therapeutic is often a CQA. This is especially true in the case of biosimilar products, where the glycosylation profile of the biosimilar product must be comparable to that of the innovator product. Known processes for performing glycan assays require a sample of a product to be manually collected, delivered to a testing laboratory, and manually concentrated, purified, and prepared for analysis. The typical turnaround time for these known, manually operated, processes is about five days. This passage of time drives costs and delays in the development of drugs (innovator and biosimilar) and eventual drug release. For example, delays of five days accumulate during drug development, for example, when optimizing culture conditions for optimal glycosylation, impeding delivery of important and new polypeptide pharmaceuticals to patients. Furthermore, such delays result in profiles determined after manufacturing, resulting in re-manufacturing products that do not meet

specifications as opposed to adjusting manufacturing parameters in real time. Thus, there is a need for efficient and faster methods to facilitate CQAs analysis, including sample preparation for such analyses.

SUMMARY

[0007] One aspect of the present disclosure provides a method including the steps of (a) moving a sample comprising molecules from a first vial to a sample loop; (b) moving a volume of the sample from the sample loop to a first multi-port valve; (c) moving the volume of the sample from the first multi-port valve to a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve; (d) when the second multi-port valve is in a first position, moving the volume of the sample from the second multi-port valve to a capture column; (e) capturing the molecules in the sample in the capture column, thereby separating the molecules in the sample from a matrix of the sample; (f) moving an elution buffer solution from a buffer source to the capture column, thereby eluting molecules captured by the capture column and moving an elution/molecule mixture comprising the elution buffer solution and the eluted molecules to a second vial arranged downstream of the capture column, the second vial comprising a flow-through vial; and (g) moving the molecules in the second vial to an analytical device for analysis of the molecules.

[0008] Another aspect of the present disclosure provides a method including the steps of: (a) moving a sample comprising polypeptides from a first vial to a sample loop; (b) moving a volume of the sample from the sample loop to a first multi-port valve; (c) moving the volume of the sample from the first multi-port valve to a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve; (d) when the second multi-port valve is in a first position, moving the volume of the sample from the second multi-port valve to a polypeptide binding column; (e) capturing the polypeptides in the sample in the polypeptide binding column, thereby separating the polypeptides in the sample from a matrix of the sample; (f) moving an elution buffer solution from a buffer source to the capture column, thereby eluting polypeptides captured by the capture column and moving an elution/molecule mixture comprising the elution buffer solution and the eluted polypeptides to a second vial arranged downstream of the polypeptide binding column, the second vial comprising a flow-through vial; and (g) moving the polypeptides in the second vial to an analytical device for analysis of the molecules. It is noted that a “vial” or “first vial” from which the sample is initially moved, as used herein, may comprise or consist of any suitable vial, such as a flow-through vial or a non-flow-through vial. Additionally, non-flow-through vials (as well as flow-through vials) may be used in methods and systems described herein as temporary or transitional containers for intermediate/partially-processed samples before they carried on to the next processing step in the system.

[0009] Another aspect of the present disclosure provides a method including the steps of: (a) moving a sample comprising polypeptides from a first vial to a sample loop; (b) moving a volume of the sample from the sample loop to a first multi-port valve; (c) moving the volume of the sample from the first multi-port valve to a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve; (d) moving the volume of the sample from the second multi-port valve to a polypeptide binding column; (e) binding the polypeptides in the volume of the sample to the polypeptide binding column, thereby separating the polypeptides in the sample from a matrix of the sample; (f) moving glycosidases (such as glucanases) to the polypeptide-binding column via the second multi-port valve to release glycans from the bound polypeptides; (g) moving the released glycans out of the polypeptide-binding column and to a second vial arranged downstream of the polypeptide-binding column, the second vial comprising a flow-through vial; (h) mixing the released glycans with a glycan-labeling reagent in the second vial; (i) moving the mixture of the

released glycans and the glycan-labeling reagent to a reaction coil via the first multi-port valve; (j) incubating the mixture of the released glycans and the glycan-labeling reagent in the reaction coil, thereby labeling the glycans; (k) moving the mixture from the reaction coil to a third vial, the third vial also comprising a flow-through vial; and (l) moving the labeled glycans in the third vial to an analytical device for analysis of the labeled glycans.

[0010] Another aspect of the disclosure provides a closed system including a first vial adapted to contain a sample comprising molecules, one or more change agents, a sample loop adapted to receive the sample from the first vial, a first multi-port valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a first port of the first multi-port valve, and a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve. The closed system includes a capture column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a first position. The capture column is configured to capture the molecules from the volume of the sample. The closed system also includes a desalting column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a second position different from the first position. The desalting column is configured to reduce a salt concentration of the molecules. The closed system further includes a first reaction coil arranged to be selectively fluidly coupled to the first multi-port valve. The first reaction coil is arranged to receive one or more of the change agents and configured to incubate the molecules and the one or more of the change agents to change the molecules. The closed system further includes a second vial arranged downstream of and fluidly coupled to the second multi-port valve. The second vial is arranged to receive a mixture comprising the molecules from the capture column, the desalting column, or the first reaction coil. The second vial includes a flow-through valve. In some examples, the flow-through vial is configured to separate the molecules from a remainder of the mixture, such that the molecules can be analyzed.

[0011] Another aspect of the disclosure provides a closed system including a first vial adapted to contain a sample comprising polypeptides, one or more change agents,

a sample loop adapted to receive the sample from the first vial, a first multi-port valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a first port of the first multi-port valve, a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve, and a capture column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a first position. The capture column is configured to capture the polypeptides from the volume of the sample. The closed system also includes a desalting column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a second position different from the first position. The desalting column is configured to reduce a salt concentration of the polypeptides. The closed system further includes a first reaction coil arranged to be selectively fluidly coupled to the first multi-port valve. The first reaction coil is arranged to receive one or more of the change agents and configured to incubate the polypeptides and the one or more of the change agents to change the polypeptides. The closed system further includes a second vial arranged downstream of and fluidly coupled to the second multi-port valve. The second vial is arranged to receive a mixture comprising the polypeptides from the capture column, the desalting column, or the first reaction coil. The second vial includes a flow-through valve configured to separate the polypeptides from a remainder of the mixture, such that the polypeptides can be analyzed.

[0012] Another aspect of the disclosure provides a closed system including a first vial adapted to contain a sample comprising polypeptides, a sample loop adapted to receive the sample from the first vial, a first multi-port valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a first port of the first multi-port valve, a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve, and a polypeptide-binding column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a first position. The polypeptide binding column is configured to bind the polypeptides from the sample. The closed system also includes a second vial arranged downstream of the polypeptide-binding

column, and a buffer source fluidly coupled to the first multi-port valve and arranged to supply elution buffer solution to the second vial via the second multi-port valve and the polypeptide-binding column when the second multi-port valve is in the first position, such that the elution buffer solution is adapted to elute substantially all of the polypeptides from the polypeptide binding column. In some examples, the second vial includes a flow-through valve configured to filter the elution buffer solution from the elution/polypeptide mixture out of the second vial, thereby leaving only the eluted polypeptides in the second vial.

[0013] Another aspect of the present disclosure provides a closed system including a first vial adapted to contain a sample comprising polypeptides, a sample loop adapted to receive the sample from the first vial, a first multi-port valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a first port of the first multi-port valve, a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve, and a polypeptide-binding column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a first position. The polypeptide binding column is configured to bind the polypeptides from the sample. The closed system also includes a second vial arranged downstream of the polypeptide-binding column, the second vial comprising a flow-through vial and comprising a glycan-labeling reagent, and a glycosidase source arranged to supply glycosidases (such as glucanase) to the polypeptide-binding column via the second multi-port valve, such that the glycosidases are infused with the polypeptides bound to the polypeptide-binding column. The closed system also includes a carrier solution source arranged to supply a carrier solution to the polypeptide-binding column via the second multi-port valve. The carrier solution is adapted to release and carry the glycans from the polypeptide-binding column and to the second vial. The closed system further includes a reaction coil arranged to receive a mixture of the released glycans and the glycan-labeling reagent from the second vial via the first multi-port valve. The reaction coil is configured to incubate the mixture of the released glycans and the glycan-labeling reagent to label the glycans. The closed system further includes a third vial arranged downstream of the reaction coil to receive the labeled glycans and the glycan-labeling reagent. In some examples, the third vial also comprises a flow-through valve configured to substantially filter the glycan-labeling reagent out of the third vial, thereby leaving substantially only the labeled glycans in the third vial.

[0014] Another aspect of the present disclosure provides a method including the steps of (a) moving a sample comprising molecules from a sample vial to a sample loop; (b) moving a volume of the sample from the sample loop to an injection valve; (c) moving the volume of the sample from the injection valve to a first multi-port valve fluidly coupled to and arranged downstream of the injection valve; (d) when the first multi-port valve is in a second position, moving the volume of the sample from the first multi-port valve to a capture column on the second multi-port valve; (e) capturing the molecules in the sample in the capture column, thereby separating the molecules in the sample from a matrix of the sample; (f) moving an elution buffer solution from a buffer source to the capture column, thereby eluting molecules captured by the capture column and moving an elution/molecule mixture comprising the elution buffer solution and the eluted molecules to a receiving vial on the third multi-port valve arranged downstream of the capture column on the second multi-port valve, the receiving vial comprising a flow-through vial; and (g) moving the molecules in the flow-through vial to an analytical device (for example, column compartment) for analysis of the molecules.

[0015] Another aspect of the present disclosure provides a method including the steps of: (a) moving a sample comprising polypeptides from a sample vial to a sample loop; (b) moving a volume of the sample from the sample loop to an injection valve; (c) moving the volume of the sample from the injection valve to a first multi-port valve fluidly coupled to and arranged downstream of the injection valve; (d) when the first multi-port valve is in a second position, moving the volume of the sample from the first multi-port valve to a polypeptide binding column on the second multi-port valve; (e) capturing the polypeptides in the sample in the polypeptide

binding column, thereby separating the polypeptides in the sample from a matrix of the sample; (f) moving an elution buffer solution from a buffer source to the capture column, thereby eluting polypeptides captured by the capture column and moving an elution/molecule mixture comprising the elution buffer solution and the eluted polypeptides to a receiving vial arranged downstream of the polypeptide binding column, the receiving vial comprising a flow-through vial; and (g) moving the polypeptides in the flow-through vial to an analytical device (for example, column compartment) for analysis of the molecules.

[0016] Another aspect of the present disclosure provides a method including the steps of: (a) moving a sample comprising polypeptides from a sample vial to a sample loop; (b) moving a volume of the sample from the sample loop to an injection valve; (c) moving the volume of the sample from the injection valve to a first multi-port valve fluidly coupled to and arranged downstream of the injection valve; (d) moving the volume of the sample from the first multi-port valve to a polypeptide binding column on the second multi-position valve; (e) binding the polypeptides in the volume of the sample to the polypeptide binding column, thereby separating the polypeptides in the sample from a matrix of the sample; (f) moving glycosidases (such as glucanase) to the polypeptide-binding column via the second multi-port valve to release glycans from the bound polypeptides; (g) moving the released glycans out of the polypeptide-binding column and to a receiving vial arranged downstream of the polypeptide-binding column, the receiving vial being a flow-through vial; (h) mixing the released glycans with a glycan-labeling reagent in the flow-through vial; (i) moving the mixture of the released glycans and the glycan-labeling reagent to a reaction coil on the second multi-port valve via the first multi-port valve; (j) incubating the mixture of the released glycans and the glycan-labeling reagent in the reaction coil, thereby labeling the glycans; (k) moving the mixture from the reaction coil to the flow-through vial; and (l) moving the labeled glycans in the flow-through vial to an analytical device for analysis of the labeled glycans.

[0017] Another aspect of the disclosure provides a closed system including a sample vial adapted to contain a sample comprising molecules, one or more change agents, a sample loop adapted to receive the sample from the sample vial, an injection valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a second port of the injection valve, and a first multi-port valve fluidly coupled to and arranged downstream of the injection valve. The closed system includes a capture column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a second position. The capture column is configured to capture the molecules from the volume of the sample. The closed system also includes a desalting column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a third position different from the second position. The desalting column is configured to reduce a salt concentration of the molecules. The closed system further includes a reaction coil arranged to be selectively fluidly coupled to the second multi-port valve. The reaction coil is arranged to receive one or more of the change agents and configured to incubate the molecules and the one or more of the change agents to change the molecules. The receiving vial is arranged to receive a mixture comprising the molecules from the capture column, the desalting column, or the reaction coil. The receiving vial includes a flow-through valve configured to separate the molecules from a remainder of the mixture, such that the molecules can be analyzed.

[0018] Another aspect of the disclosure provides a closed system including a sample vial adapted to contain a sample comprising polypeptides, one or more change agents,

a sample loop adapted to receive the sample from the first vial, an injection valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a second port of the injection valve, a first multi-port valve fluidly coupled to and arranged downstream of the injection valve, and a capture column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a second position. The capture column is configured to capture the polypeptides from the volume of the sample. The closed system also includes a desalting column arranged to be fluidly coupled to the second multi-port valve when the

second multi-port valve is in a third position different from the second position. The desalting column is configured to reduce a salt concentration of the polypeptides. The closed system further includes a reaction coil arranged to be selectively fluidly coupled to the second multi-port valve. The reaction coil is arranged to receive one or more of the change agents and configured to incubate the polypeptides and the one or more of the change agents to change the polypeptides. The closed system further includes a receiving vial arranged downstream of and fluidly coupled to the third multi-port valve. The receiving vial is arranged to receive a mixture comprising the polypeptides from the capture column, the desalting column, or the first reaction coil. The receiving vial includes a flow-through valve configured to separate the polypeptides from a remainder of the mixture, such that the polypeptides can be analyzed.

[0019] Another aspect of the disclosure provides a closed system including a sample vial adapted to contain a sample comprising polypeptides, a sample loop adapted to receive the sample from the sample vial, an injection valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a second port of the injection valve, a first multi-port valve fluidly coupled to and arranged downstream of the injection valve, and a polypeptide-binding column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a second position. The polypeptide binding column is configured to bind the polypeptides from the sample. The closed system also includes a receiving vial on the third multi-port valve arranged downstream of the polypeptide-binding column on the second multi-port valve. A buffer source fluidly coupled to the first multi-port valve and arranged to supply elution buffer solution to the second multi-port valve and the polypeptide-binding column when the second multi-port valve is in the second position, such that the elution buffer solution is adapted to elute substantially all of the polypeptides from the polypeptide binding column into the receiving vial on the third multi-position valve The receiving vial includes a flow-through valve configured in combination of the third multi-port valve, to divert the elution buffer solution from the elution/polypeptide mixture out of the polypeptide-binding column, thereby leaving only the eluted polypeptides in the flow-thru vial.

[0020] Another aspect of the present disclosure provides a closed system including a sample vial adapted to contain a sample comprising polypeptides, a sample loop adapted to receive the sample from the first vial, an injection valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a second port of the injection valve, a first multi-port valve fluidly coupled to and arranged downstream of the injection valve, and a polypeptide-binding column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in the second position. The polypeptide binding column is configured to bind the polypeptides from the sample. The closed system also includes a receiving vial on the third multi-port valve arranged downstream of the polypeptide-binding column, the receiving vial being a flow-through vial. comprising a glycan-labeling reagent, and a glycosidase source arranged to supply glycosidases (such as glucanase) to the polypeptide-binding column via the second multi-port valve, such that the glycosidases are infused with the polypeptides bound to the polypeptide-binding column. The closed system also includes a carrier solution source arranged to supply a carrier solution to the polypeptide-binding column via the second multi-port valve. The carrier solution is adapted to release and carry the glycans from the polypeptide-binding column and to the receiving vial. The closed system further includes a reaction coil arranged to receive a mixture of the released glycans and the glycan-labeling reagent from the flow-through vial via the first multi-port valve. The reaction coil is configured to incubate the mixture of the released glycans and the glycan-labeling reagent to label the glycans. The closed system uses the same flow-thru vial on the third multi-port valve arranged downstream of the reaction coil on the second multi-port valve to divert the glycan-labeling reagent but to receive the labeled glycans in the flow-through vial.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1 is a schematic diagram of one example of a system for performing an online, real-time assay assembled in accordance with the teachings of the present disclosure.

[0022] FIG. 2 is a schematic diagram of a controller of the system illustrated in FIG. 1.

[0023] FIG. 3 is a schematic diagram illustrating one example of a method for performing a real-time assay of a sample using the system of FIG. 1.

[0024] FIG. 4 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 1.

[0025] FIG. 5 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 1.

[0026] FIG. 6 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 1.

[0027] FIG. 7 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 1.

[0028] FIG. 8 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 1.

[0029] FIG. 9 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 1.

[0030] FIG. 10 is a schematic diagram of another example of a system for performing an online, real-time assay assembled in accordance with the teachings of the present disclosure.

[0031] FIG. 11 is a schematic diagram illustrating one example of a method for performing a real-time assay of a sample using the system of FIG. 10.

[0032] FIG. 12 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 10.

[0033] FIG. 13 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 10.

[0034] FIG. 14 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 10.

[0035] FIG. 15 is a schematic diagram illustrating another example of a method for performing a real-time assay of a sample using the system of FIG. 10.

[0036] FIG. 16 is a perspective view of one example of a flow-through vial that is constructed in accordance with the teachings of the present disclosure and can be used in the system of FIG. 1 or the system of FIG. 10.

[0037] FIG. 17 is a partial, cross-sectional view of the flow-through vial of FIG. 16.

[0038] FIG. 18 is a perspective view of one example of a vial holder that is constructed in accordance with the teachings of the present disclosure and can be used in the system of FIG. 1 or the system of FIG. 10 to hold a plurality of the flow-through vials of FIG. 16.

[0039] FIG. 19 illustrates the vial holder coupled to an automated sampling system of the system of FIG. 1 or the system of FIG. 10 and receiving one of the flow-through vials of FIG. 16.

[0040] FIG. 20 is a table depicting the results of a study validating the effectiveness of the system of FIG. 1 using three different assays and nine different molecules.

[0041] FIG. 21 is a graph depicting the results of one of the three different assays performed with one of the nine different molecules using the system of FIG. 1.

[0042] FIG. 22 is similar to FIG. 21, but shows the results using a conventional technique.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0043] FIG. 1 illustrates a schematic diagram of an example of a system 100 assembled in accordance with the teachings of the present disclosure. The system 100, which can be located at or in a laboratory (*e.g.*, a testing laboratory) or in a manufacturing facility (e.g., on the manufacturing floor), is a closed system for automatically or substantially automatically preparing a sample of a product containing molecules for analysis and automatically performing an assay of that sample, as is described in greater detail below. By automating (or substantially automating) this process using the system 100, the assay can be performed in real-time (or substantially in real-time) and on the manufacturing floor (when used in the manufacturing facility), such that the entire process can be performed, and the desired result obtained, in a matter of hours (*e.g.*, 2 to 3 hours), a significant improvement over the 5 days typically required by the conventionally known, manually-operated processes. Moreover, the closed nature of the process that utilizes the system 100 maintains sterile conditions. Further yet, the system 100 is a flexible system that can be used to prepare any number of samples (the same or different) for different analyses and can automatically perform a number of different assays on these samples, thereby obviating the need for multiple different systems for preparing different samples for different analyses. By performing multiple assays within the same system 100, less volume of sample may be required than if the same assays were performed on multiple systems (which would typically result in loss of some sample as dead volume). Thus, it is contemplated that systems 100 described herein can conserve sample by minimizing or avoiding dead volume. Moreover, systems 100 as described herein can be capable of performing multiple assays while maintaining a smaller footprint than individual systems configured to perform each assay individually. For example, a system as described herein equipped with a capture column (such as a protein A column) in valve 112 can achieve the functionality of a larger-scale purification but use a smaller footprint.

[0044] The system 100 illustrated in FIG. 1 generally includes one or more sample vials, a needle, a sample loop, a metering device, a needle seat port, a first column, a second column, one or more buffer sources, one or more sources of change reagents, one or more sources of carrier solution(s), a first reaction coil (Coil A in FIG. 1), a second reaction coil (Coil B in FIG. 1), and one or more receiving vials, as well as four multi-port valves 104, 108, 112, 116 and conduit (e.g., stainless steel conduit) extending between each of the components of the system 100 so as to facilitate fluid communication between components of the system 100 when desired. The system 100 illustrated in FIG. 1 also includes an analytical device, a first pump 120, and a second pump 124. It will nonetheless be appreciated that the system 100 need not include one or more of the above-listed components and/or can include additional components. Optionally, the second reaction coil (e.g., Coil B in FIG. 1) may be omitted from the system 100.

[0045] Each of the one or more sample vials is adapted to contain a sample including molecules of interest. The sample is preferably obtained (*e.g.*, delivered) automatically using an automatic sampling system (*e.g.*, the autosampler employed in the Agilent 1290 Infinity II product), though the sample can be obtained manually. In some examples, the molecules are polypeptides, such as,

for example, therapeutic polypeptides (discussed in further detail below) and polynucleotides. In other examples, the molecules are small
molecules (*i.e.*, having a molecular weight of approximately 900 Daltons or less and can diffuse across cell membranes within a reasonable amount of time). The small molecules can, for example, comprise, consist essentially of, or consist of metabolites. Each of the one or more sample vials is preferably housed in the automatic sampling system itself and generally takes the form of a flow-through vial, details about which are described in greater detail herein, though the sample vials can be other vials *(e.g.*, non-flow-through vials) as well. In any event, when desired, the needle is movable to automatically retrieve a sample from one of the sample vials and deposit the sample into the sample loop. The sample loop serves as a temporary reservoir or buffer zone for the deposited sample and, at the same time, prevents the sample from entering the metering device. The metering device, which in this example takes the form of an analytical head, is arranged to control the volume of the sample obtained by the needle and deposited into the sample loop. The sample is ultimately pushed from the sample loop into the needle seat port.

[0046] The multi-port valve 104 in this example takes the form of a 6-port injection valve. When desired, the multi-port valve 104 is movable into fluid communication with the needle seat port. In turn, the sample will flow from the needle seat port through the multi-port valve 104 and to another, downstream component of the system 100 (the specific component will depend on the assay being performed). In some cases, the sample will flow from the needle seat port through the multi-port valve 104 and to the analytical device. In some cases, the sample will flow from the needle seat port through the multi-port valve 104 and to the multi-port valve 108.

[0047] The multi-port valve 108 in this example takes the form of a 6-port injection valve. The multi-port valve 108 is in selective fluid communication with the multi-port valve 104, such that the sample (and/or buffer(s) from the buffer source(s) or change reagent(s) from the change reagent source(s)) can flow from the needle seat port to the multi-port valve 108 via the multi-port valve 104.

[0048] The multi-port valve 112 in this example is a 4-position 10-port valve, the position of which determines whether the multi-port valve 108 is fluidly coupled to the first column or the second column (both of which are downstream of the multi-port valve 108). When the multi-port valve 112 is in a first position, the first column is fluidly coupled to the multi-port valve 108 (and in turn the multi-port valve 104) and the second column is fluidly isolated from the multi-port valve 108, such that the first column can receive the sample, the buffer(s), the change reagent(s), the carrier solution(s), or combinations thereof. Conversely, when the multi-port valve 112 is in a second position different from the first position, the second column is fluidly coupled to the multi-port valve 108 (and in turn the multi-port valve 104) and the first column is fluidly isolated from the multi-port valve 108, such that the second column can receive the sample, the buffer(s), the change reagent(s), and/or the carrier solution(s). It is contemplated that the multi-port valve 112 of systems and methods described herein can be configured to perform an analytical assay, such as a column-based assay. In systems and methods described herein, the first column can comprise or consist of an analytical column. For example, the analytical column can be a column of a chromatography device as described herein, or an incubation column. The incubation column may be configured for protein digestion in accordance with a MAM as described herein.

[0049] The first column in this example is a capture column configured to capture the molecules of interest from the sample, thereby substantially separating the molecules in the sample from a matrix of the sample (which can in turn be passed to waste). In some cases, the capture column can also serve to pre-concentrate the molecules (*e.g.,* by clustering them in the capture column), though this step is not necessary. In cases in which the molecules are polypeptides, the capture column takes the form of a polypeptide-binding column selected from a group consisting of a protein A column, a protein G column, a protein A/G column, a protein L column, an amino acid column, an avidin column, a streptavidin column, a carbohydrate bonding column, a carbohydrate

column, a glutathione column, a heparin column, a hydrophobic interaction column, an immunoaffinity column, a nucleotide/coenzyme column, a specialty column, and an immobilized-metal affinity chromatography (IMAC) column. For example, in the case of polypeptides that are human IgGs of subclasses 1, 2, or 4, IgM, IgA, or IgE (and comprising a human Fc portion and/or a Fab region of the human VH3 family), protein A columns are useful. Protein G can be used to purify human IgGs of subclasses 1-4. Recombinant fusion protein A/G can also be used to purify all of these classes of human antibodies, as the fusion protein provides protein A and protein G binding sites. Thus, protein A/G fusion proteins can be used to purify human IgG, IgA, IgE, and IgM. Furthermore, protein L can be used to purify human IgG, IgM, IgA, IgE and IgD, provided the target antibodies have an appropriate kappa (κ) subtype light chain (*i.e.*, VκI, VκIII and VκIV subtypes); protein L can also be used to purify Fab and scFv fragments also having the appropriate κ chain subtype, as protein L binds the variable (V) chain of antibodies. However, in cases in which the molecules are small molecules, the capture column can instead take the form of a reverse phase column, a size exclusion column, an ion exchange column, a normal phase column, a chiral separation column, a mix mode column, or a hydrophobic interaction column. For example, in cases in which the molecules are small molecules such as metabolites, the capture column can take the form of a tri-mode (or tri-phase) chromatography column that includes reverse phase, cationic (cation-exchange) and anionic (anion exchange). Analytes may be eluted by pH, ionic strength, and/or organic strength gradients. The elution buffer for the analysis of small molecules (such as metabolites) may comprise aqueous, low-salt volatile buffer. In some examples, the low-salt volatile buffer further comprises an organic solvent. Levels of the organic solvent may be lower than organic solvent levels used in HILIC. By way of example, a typical starting solvent for typical HILIC may have an organic concentration of ≥ 50% (v/v).

[0050] Meanwhile, the second column in this example is a desalting column equilibrated with a buffer (*e.g.*, a proteolysis buffer), such that the second column is configured to reduce a salt concentration of the molecules. Thus, when, for example, the molecules are polypeptides, the desalting column is configured to reduce a salt concentration of the polypeptides (*i.e.*, desalt the polypeptides). The second column preferably takes the form of a size exclusion chromatography column, though other chromatography columns can be used instead.

[0051] The needle is also movable to obtain buffer solution(s) from the one or more buffer sources and deposit the buffer solution(s) in the sample loop. In turn, the buffer solution(s) can flow to the multi-port valve 108 via the multi-port valve 104 in a similar manner as described above (in connection with the flow of the sample). The buffer solution(s) can subsequently be directed to the appropriate component in the system 100. In one example, the buffer solution can be an elution buffer solution (*e.g.*, a volatile buffer (*e.g.*, a low-salt volatile buffer, such as water + formic acid; water and acetonitrile + ammonium acetate; or water and acetonitrile + ammonium bicarbonate), in the case of protein A-bound antibodies, an acidic buffer, or in the case of protein G-bound antibodies, a very acidic (pH 3 or less) buffer) that is ultimately supplied to the first column when the multi-port valve 112 is in the first position. As the elution buffer solution flows to and through the first column, the elution buffer solution elutes substantially all of the molecules (*e.g.*, the polypeptides) captured in the first column.

[0052] The needle is also movable to obtain change reagent(s) from the one or more sources of change reagents and deposit the change reagent(s) in the sample loop. In turn, the change reagent(s) can flow to the multi-port valve 108 via the multi-port valve 104 in a similar manner as described above (in connection with the flow of the sample). The change reagent(s) can subsequently be directed to the appropriate component in the system 100 in order to change the molecules in the desired manner.

[0053] Generally speaking, the change reagent(s) are selected from the group consisting of a denaturing reagent, a reducing reagent, an alkylating reagent, a solution containing (*e.g.*, consisting of) glycosidases (such as glucanase), a glycan labeling reagent, a quenching reagent, an enzyme, and combinations thereof.

[0054] A denaturing reagent can be utilized to denature the molecules (*e.g.*, the polypeptides) in the sample. In examples in which the change reagent(s) is or includes a denaturing reagent, the denaturing reagent can be or include a denaturing detergent or a chaotrope. In those examples in which the denaturing reagent is or includes a denaturing detergent, the denaturing detergent is preferably selected from the group consisting of sodium dodecyl sulfate (SDS), sodium cholate, sodium deoxycholate, sodium glycocholate, sodium taurocholate, sodium taurodeoxycholate, N-lauroylsarcosine, lithium dodecyl sulfate, hexadecyltrimethyl ammonium bromide (CTAB) and trimethyl(tetradecyl) ammonium bromide (TTAB). More preferably, the denaturing detergent is SDS. In those versions in which the denaturing reagent is or includes a chaotrope, the chaotrope is preferably selected from the group consisting of urea, n-butanol, ethanol, guanidinium chloride, lithium perchlorate, lithium acetate, magnesium chloride, phenol, 2-propanol, and thiourea. Alternatively or additionally, the denaturing reagent can be or include a heated fluid that has a temperature suitable for reaching, if not maintaining, a pre-determined temperature (*e.g.*, about 22 °C to about 120 °C).

[0055] A reducing reagent can be utilized to cleave disulfide bond crosslinks, thereby reducing the molecules (*e.g.*, the polypeptides). The reducing reagent can be selected from the group consisting of dithiothreitol (DTT), glutathione, β-mercaptoethanol (β-ME), and tris(2-carboxyethyl)phosphine (TCEP). While not illustrated herein, the reducing reagent can be supplied by a cooling vessel (*e.g.,* a chiller having a temperature of 4 °C). Meanwhile, when utilized, an alkylating agent alkylates sulfhydryls, thereby alkylating the molecules (*e.g.*, the polypeptides). The alkylating agent is preferably an alkylating reagent such as indole-3-acetic acid (IAA), though other alkylating agents can be used.

[0056] A solution containing glycosidases can be utilized to direct glycosidases to and infuse glycosidases with the molecules (*e.g.*, the polypeptides) captured by the first column, which in turn releases glycans in the captured molecules (*e.g.*, the polypeptides) from the captured molecules (*e.g.*, the polypeptides) into the solution. The glycosidases in the solution are preferably selected from the group consisting of glucanase, endoglycosidases, glycosamidases, and O-glycanases, and combinations thereof (for example, endoglycosidases, glycosamidases, and O-glycanases, and combinations thereof). When the glycosidases are or include endoglycosidases, the endoglycosidases are preferably selected from the group consisting of endoglycosidase D, endoglycosidase F (endoglycosidase F1, endoglycosidase F2, and endoglycosidase F3 and combinations thereof), endoglycosidase H, endoglycosidase S, endoglycosidase M, and endoglycosidase B. When the glycosidases are or include glycosamidases, the glycosamidases are preferably selected from the group consisting of glycopeptidases, peptide N-glycosidases, PNGases, N-glycohydrolases, and N-glycanases. When the glycosidases are or include PNGases, the PNGases preferably include peptide:N-glycosidase F (PNGF). When the glycosidases are or include O-glycanases, the O-glycanases preferably are endo-GalNAc-ase D or endoGalNAc-ase A.

[0057] When glycans are released into the solution in this manner, the needle is further movable to obtain a carrier solution (*e.g.*, de-ionized (DI) water) from the one or more sources of carrier solutions and deposit the carrier solution in the sample loop. In turn, the carrier solution can flow to the multi-port valve 108 via the multi-port valve 104 in a similar manner as described above (in connection with the flow of the sample). The carrier solution can, for example, subsequently be supplied to and through the first column to carry the released glycans from the first column.

[0058] In turn, a glycan labeling reagent can be utilized to label (*e.g.,* fluorescently label) glycans that have been released from the molecules (*e.g.*, the polypeptides) and carried away from the first column. The glycan labeling reagent preferably takes the form of, for example, a fluorophore (*e.g.*, selected from the group consisting of 2-aminobenzoic acid, 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt, 8-aminonaphthalene-1,3,6-trisulfonic acid trisodium salt, and anthranilamide, 4-methoxybenzamidine) or a chromophore (*e.g.*, 3-methyl-1-phenyl-2-pyrazoline-5-one or phenylhydrazine).

[0059] An enzyme can be utilized to digest the molecules (*e.g.*, the polypeptides). The enzyme preferably takes the form of a protease, cellulase, lipase, amylase, glucoamylase, glucose isomerase, xylanase, phytase, arabinanase, polygalacturonanase, hydrolase, chymosin, urease, pectinase, or beta-glucanase. A quenching reagent, meanwhile, can be utilized to stop a reaction (e.g., an enzymatic reaction) by altering the pH condition of the sample. The quenching reagent preferably takes the form of trifluoroacetic acid (TFA), though other quenching reagents can be utilized instead.

[0060] The multi-port valve 116 in this example is a 4-position 10-port valve, the position of which determines whether the multi-port valve 108 is fluidly coupled to the first reaction coil or the second reaction coil (both of which are downstream of the multi-port valve 108). When the multi-port valve 116 is in a first position, the first reaction coil is fluidly coupled to the multi-port valve 108 (and in turn the multi-port valve 104) and the second reaction coil is fluidly isolated from the multi-port valve 108, such that the first reaction coil can receive the sample or portions thereof (e.g., glycans released from the polypeptides in the sample), the buffer(s), the change reagent(s), the carrier solution(s), or combinations thereof. Conversely, when the multi-port valve 116 is in a second position different from the first position, the second reaction coil is fluidly coupled to the multi-port valve 108 (and in turn the multi-port valve 104) and the first reaction coil is fluidly isolated from the multi-port valve 108, such that the second reaction coil can receive the sample or portions thereof, the buffer(s), and/or the change reagent(s).

[0061] Each of the first and second reaction coils is configured to incubate the molecules (*e.g.*, the polypeptides) and one or more of the change agents to change the molecules (*e.g.*, the polypeptides). The first or second reaction coil can, for example, receive a quenching reagent and the polypeptides and incubate the quenching reagent and the polypeptides in order to facilitate quenching of the polypeptides. The first or second reaction coil can, as another example, receive a denaturing reagent and the polypeptides and incubate the denaturing reagent and the polypeptides in order to facilitate denaturing of the polypeptides. The first or second reaction coil can, as another example, receive a reducing reagent and the polypeptides and incubate the reducing reagent and the polypeptides in order to facilitate reduction of the polypeptides. The first or second reaction coil can, as yet another example, receive an alkylating reagent and the polypeptides and incubate the alkylating reagent and the polypeptides in order to facilitate alkylation of the polypeptides. The first or second reaction coil can, as a further example, receive a mixture including released glycans and a glycan-labeling reagent and incubate the mixture in order to facilitate labeling (*e.g.*, fluorescently labeling) of the released glycans.

[0062] In order to facilitate the desired incubation, the system 100 can further include a heating element positioned immediately adjacent or otherwise thermally coupled to the first and/or second reaction coils. The heating element can, for example, take the form of a heating coil, an induction heater, a heat pump, a cartridge heater, an electrical resistance wire, or other element suitable for heating one or more portions of the respective first or second reaction coil. Thus, when the system 100 includes a heating element thermally coupled to the first reaction coil, the heating element is configured to maintain the first reaction coil at a first pre-determined incubation temperature, which can, for example, be approximately 30 °C, approximately 40 °C, approximately 80 °C, or some other incubation temperature, depending upon the application. Similarly, when the system 100 includes a heating element thermally coupled to the second reaction coil, the heating element is configured to maintain the second reaction coil at a second pre-determined incubation temperature, which can be the same as or different than the first pre-determined incubation temperature.

[0063] The one or more receiving vials in this example are generally arranged downstream of all of the other components of the system 100. Like the one or more sample vials, the one or more receiving vials generally take the form of one or more flow through vials, though other vials (*e.g.*, non-flow-through vials) can be used as well. The one or more receiving vials are generally fluidly coupled to the multi-port valve 112 and/or the multi-port valve 116. The one or more receiving vials are therefore generally arranged

to receive a mixture including the molecules (*e.g.*, the polypeptides) from one or more of the first column, the second column, the first reaction coil, and the second reaction coil. In the illustrated example, the system 100 utilizes two flow-through vials, a first one that is fluidly coupled to the multi-port valve 112 and a second one that is fluidly coupled to the multi-port valve 116. Thus, the first flow-through vial is arranged to receive a mixture including the molecules from the first column or the second column (depending upon which column is being used). The mixture can, for example, include the elution buffer solution and molecules eluted by the elution buffer solution from the first column. On the other hand, the second flow-through vial is arranged to receive a mixture including the molecules from the first reaction coil or the second reaction coil (depending upon which coil is being used). The mixture can, for example, include molecules that were denatured and reduced in the first reaction coil.

[0064] In some examples, it will be appreciated that each of the receiving vials can have a plurality of holes that are arranged and sized to substantially prevent the molecules in the received mixture from passing therethrough but allow a remainder of the mixture to pass therethrough and out of the respective receiving vial. Thus, the holes in each of the receiving vials effectively separate the molecules in the received mixture from a remainder of the mixture. The separated molecules can, in turn, be passed to the analytical device (for analysis of the molecules) or can be passed to other components in the system 100 for further preparation. Meanwhile, the remainder of the mixture can, in turn, be passed to waste. Alternatively or additionally, this separation function can be performed by timely moving one or more of the multi-port valve 104, the multi-port valve 108, the multi-port valve 112, and the multi-port valve 116.

[0065] In some examples, such as the one illustrated in FIG. 1, the system 100 can also include one or more intermediate vials each including a fluid configured to dilute the salt concentration in the sample. The fluid can, for example, take the form of water, a buffer (e.g., a low salt buffer), an organic solvent (*e.g.*, methanol, ethanol, propanol, acetone) or a mobile phase that serves as a diluent. In any event, when it is necessary to dilute the salt concentration in the sample, the needle is movable to either (i) obtain the sample from the appropriate component in the system 100 and deposit the sample in the respective intermediate vial for dilution, or (ii) obtain the fluid from the respective intermediate vial and deposit the fluid in the component of the system 100 containing the sample.

[0066] The first pump 120 in this example takes the form of a binary or quaternary pump that is fluidly coupled to the multi-port valve 104. The binary or quaternary pump is generally arranged to help move materials through the multi-port valve 104 and to other components in the system 100. In this example, the pump is arranged to drive samples from the needle seat port and drive mobile phases to the first column, the second column, or other component of the system 100, as desired.

[0067] The second pump 124 in this example takes the form of a quaternary pump that is fluidly coupled to the multi-port valve 108. The second pump 124 is generally arranged to help clean various components of the system 100. In this example, the second pump 124 is arranged to drive various solutions (e.g., buffers) to the first and second columns in order to clean and equilibrate these columns, and to drive various solutions to the first and second reaction coils to flush the coils in order to prevent carryover from sample to sample. For example, the second pump 124 may be configured to flush the flow-through vial *in situ*, so that the flow-through vial does not need to be removed for flushing, thus simplifying the design of the system 100 and minimizing the number of moving parts in the system 100.

[0068] The analytical device is generally configured to analyze the molecules (*e.g.*, the polypeptides) after the molecules (*e.g.*, the polypeptides) have been prepared by the system 100. The analytical device can, for example, take the form of a liquid chromatography device (*e.g.*, an ion exchange chromatography column, a cation exchange chromatography column, an anion-exchange chromatography column), a high-performance liquid chromatography device, an ultra-high-performance liquid

chromatography device, a mass spectrometry device (*e.g.*, a high-resolution accurate-mass (HRAM) mass spectrometer), a spectrophotometric device (*e.g.*, a UV detector), a glycan analysis device, another type of analytical device, or a combination thereof. It is contemplated that a HRAM mass spectrometer can be useful for the analysis of small molecules such as metabolites as described herein. It will also be appreciated that multiple analytical devices can be employed in the system 100.

[0069] As illustrated in FIG. 1, the system 100 also includes a controller, which in this example is communicatively coupled or connected to various components of the system 100 to monitor and facilitate or direct the above-described operation of the system 100 by transmitting signals (*e.g.*, control signals, data) to and receiving signals (*e.g.*, data) from the various components of the system 100. The controller can be located immediately adjacent the other components of the system 100 (*e.g.*, in the same environment as the system 100) or can be remotely located from the other components of the system 100.

[0070] As used herein, the phrases "communicatively coupled" and "connected" are defined to mean directly coupled or connected to or indirectly coupled or connected through one or more intermediate components. Such intermediate components can include hardware and/or software-based components. It is appreciated that the controller can be communicatively coupled or connected to various components of the system 100 via one or more wireless networks, one or more wired networks, one or more combinations of a wired and a wireless network (*e.g.*, a cellular telephone network and/or 802.11x compliant network), and can include a publicly accessible network, such as the Internet, a private network, or a combination thereof. The type and configuration of the networks is implementation dependent, and any type of communications networks which facilitate the described communications between the controller and the components of the system 100, available now or later developed, can be used.

[0071] As shown in FIG. 2, the controller includes a processor 352, a memory 356, a communications interface 360, and computing logic 364. The processor 352 can be a general processor, a digital signal processor, ASIC, field programmable gate array, graphics processing unit, analog circuit, digital circuit, or any other known or later developed processor. The processor 352 operates pursuant to instructions in the memory 356. The memory 356 can be a volatile memory or a non-volatile memory. The memory 356 can include one or more of a read-only memory (ROM), random-access memory (RAM), a flash memory, an electronic erasable program read-only memory (EEPROM), or other type of memory. The memory 356 can include an optical, magnetic (hard drive), or any other form of data storage device.

[0072] The communications interface 360 is provided to enable or facilitate electronic communication between the controller and the components of the system 100 via the one or more utilized networks. The communications interface 360 can be or include, for example, one or more universal serial bus (USB) ports, one or more Ethernet ports, and/or one or more other ports or interfaces. The electronic communication can occur via any known communications protocol, including, by way of example, USB, RS-232, RS-485, WiFi, Bluetooth, and/or any other suitable communications protocol.

[0073] The logic 364 generally includes one or more control routines and/or one or more sub-routines embodied as computer-readable instructions stored on the memory 356. The control routines and/or sub-routines can perform PID (proportional-integral-derivative), fuzzy logic, nonlinear, or any other suitable type of control. The processor 352 generally executes the logic 364 to perform actions related to the operation of the system 100.

[0074] Generally speaking, the logic 364, when executed, causes the processor 352 to control components of the system 100, particularly the multi-port valves 104, 108, 112, 116, the needle, the pumps 120, 124, and the heating element(s), such that the system 100 operates in the desired manner discussed herein. As an example, the logic 364 can, when executed, cause the

processor 352 to move the multi-port valve 112 and/or the multi-port valve 116 to or between any of the positions described herein, thereby fluidly coupling various components of the system 100 as described above.

[0075] In other versions, the logic 364 can, when executed by the processor 352, cause additional, less, and/or different functionality to be performed. Moreover, in other versions, the logic 364 can be executed by the processor 352 in a different order than described herein. Finally, it is appreciated that the logic 364 can be executed by the processor 352 any number of different times, as the system 100 can be used to perform real-time analyses of multiple samples (from the same product and/or from a different product).

[0076] FIG. 3 illustrates an example of a method 300 of automatically (or substantially automatically) performing a first real-time assay of a sample using the system 100. In this example, the assay is a production titer measurement and the method 300 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample from one of the sample vials and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the analytical device, which in this example takes the form of a spectrophotometric detector, via the multi-port valve 104, and (4) determining the titer / concentration of the sample using the analytical device.

[0077] FIG. 4 illustrates another example of a method 400 of automatically (or substantially automatically) performing a second real-time assay of a sample using the system 100. In this example, the assay is an aggregation assessment and the method 400 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to the first column via the multi-port valve 112 (which is in the first position), (5) capturing the molecules in the sample in the first column, thereby separating the molecules in the sample from a matrix of the sample, which flows out of the first column and to waste via a second vial (one of the receiving vials) fluidly coupled thereto, (6) moving an elution buffer solution from a buffer source to the first column via the needle, the needle seat port, and the multi-port valves 104, 108, and 112, thereby eluting molecules captured by the first column, (7) moving an elution / molecule mixture including the elution buffer solution and the eluted molecules to a second vial (one of the receiving vials), which separates the molecules in the mixture from a remainder of the mixture, (8) moving the molecules from the second vial to the analytical device via the needle, the needle seat port, and the multi-port valve 104, and (9) quantifying the level of aggregation in the molecules using the analytical device.

[0078] FIG. 5 illustrates another example of a method 500 of automatically (or substantially automatically) performing a third real-time assay of a sample using the system 100. In this example, the assay is an charge variant profile and the method 500 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to the first column via the multi-port valve 112 (which is in the first position), (5) capturing the molecules in the sample in the first column, thereby separating the molecules in the sample from a matrix of the sample, which flows out of the first column and to waste via a second vial (one of the receiving vials) fluidly coupled thereto, (6) moving an elution buffer solution from a buffer source to the first column via the needle, the needle seat port, and the multi-port valves 104, 108, and 112, thereby eluting molecules captured by the first column, (7) moving an elution / molecule mixture including the elution buffer solution and the eluted molecules to a second vial (one of the receiving vials), which separates the molecules in the mixture from a remainder of the mixture, thereby forming an affinity purified sample in the second vial, (8) moving the molecules from the second vial to a third vial (one of the intermediate vials)

to dilute the affinity purified sample, (9) moving the affinity purified sample from the third vial back to the multi-port valve 108 via the needle, the needle seat port, and the multi-port valve 104, (10) moving the affinity purified sample from the multi-port valve 108 to the second column via the multi-port valve 112 (which has been moved from the first position to the second position), (11) applying the affinity purified sample to the second column, thereby further reducing the salt concentration of the sample, (12) moving the further diluted affinity purified sample from the second column back to the second vial, (13) moving the affinity purified sample from the second vial to the analytical device via the needle, the needle seat port, and the multi-port valve 104, and (14) performing a charge variant assessment using the analytical device (which in this example takes the form of an ion exchange column).

[0079] FIG. 6 illustrates another example of a method 600 of automatically (or substantially automatically) performing a fourth real-time assay of a sample using the system 100. In this example, the assay is a glycosylation profiling assay and the method 600 generally includes: (1) moving (*e.g.*, automatically moving) the needle to obtain a sample from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to the first column via the multi-port valve 112 (which is in the first position), (5) capturing the molecules in the sample in the first column, thereby separating the molecules in the sample from a matrix of the sample, which flows out of the first column and to waste via a second vial (one of the receiving vials) fluidly coupled thereto, (6) moving a solution containing glycosidases (in this example PNGase-F) from a source to the first column via the needle, the needle seat port, and the multi-port valves 104, 108, and 112, thereby releasing glycans from the molecules captured by the first column, (7) moving the released glycans to a second vial (one of the receiving vials), (8) moving a glycan-labeling reagent (in this example 2-AA) from a source to the second vial via the needle, (9) mixing the glycan-labeling reagent and the released glycans in the second vial, (10) moving the mixture of the released glycans and the glycan-labeling reagent to the second reaction coil (coil B) via the needle, the multi-port valves 104, 108, and the multi-port valve 112 (which is in the fourth position), (11) incubating the mixture in the second reaction coil, thereby fluorescently labeling the glycans, (12) moving the mixture from the second reaction coil to a third vial (another one of the receiving vials), which separates the labeled glycans in the mixture from a remainder of the mixture, (13) moving the labeled glycans from the third vial to the analytical device via the needle, the needle seat port, and the multi-port valve 104, and (14) performing normal-phase chromatographic separation and quantitation using the analytical device.

[0080] FIG. 7 illustrates another example of a method 700 of automatically (or substantially automatically) performing a fifth real-time assay of a sample using the system 100. In this example, the assay is a MAM assay and the method 700 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to the first column via the multi-port valve 112 (which is in the first position), (5) capturing the molecules in the sample in the first column, thereby separating the molecules in the sample from a matrix of the sample, which flows out of the first column and to waste via a second vial (one of the receiving vials) fluidly coupled thereto, (6) moving an elution buffer solution from a buffer source to the first column via the needle, the needle seat port, and the multi-port valves 104, 108, and 112, thereby eluting molecules captured by the first column, (7) moving an elution/molecule mixture including the elution buffer solution and the eluted molecules to a second vial (one of the receiving vials), which separates the molecules in the mixture from a remainder of the mixture, thereby forming an affinity purified sample in the second vial, (8) moving, via the needle, the affinity purified sample from the second vial to a third vial (one of the intermediate vials) to dilute the affinity purified sample, (9) moving the diluted affinity purified sample from the third vial back to the

second vial via the needle, (10) moving a denaturing reagent and a reducing reagent to the second vial via the needle, (11) moving the affinity purified sample, the denaturing reagent, and the reducing reagent from the second vial to the first reaction coil (coil A) via the needle, the multi-port valves 104, 108, and the multi-port valve 112 (which is in the third position), (12) incubating the mixture in the first reaction coil, thereby denaturing and reducing the molecules, (13) moving the denatured and reduced molecules from the first reaction coil to a fourth vial (another one of the receiving vials) fluidly coupled to the first reaction coil, (14) moving an alkylating reagent to the fourth vial via the needle, (15) moving the denatured and reduced molecules and the alkylating reagent from the fourth vial to the first reaction coil (coil A) via the needle, the multi-port valves 104, 108, and the multi-port valve 112 (which is in the third position), (16) incubating the denatured and reduced molecules and the alkylating reagent in the first reaction coil, thereby alkylating the denatured and reduced molecules, (17) moving the denatured, reduced, and alkylated molecules from the first reaction coil to the fourth vial, (18) moving the denatured, reduced, and alkylated molecules from the fourth vial to the second column via the needle, the needle seat port, the multi-port valves 104, 108, and the multi-port valve 112 (which has been moved from the first position to the second position), (19) applying the denatured, reduced, and alkylated molecules to the second column, thereby reducing the salt concentration of the molecules, (20) moving the desalted molecules from the second column back to the second vial, (21) moving an enzyme to the second vial via the needle (either before step (20) or after step (20), (22) moving the desalted molecules and the enzyme from the second vial to the second reaction coil (coil B) via the needle, the multi-port valves 104, 108, and the multi-port valve 112 (which has been moved to the fourth position), (23) incubating the desalted molecules and the enzyme in the second reaction coil, thereby digesting the molecules, (24) moving the digested molecules from the second reaction coil back to the fourth vial, (25) moving the digested molecules from the fourth vial to the analytical device via the needle, the needle seat port, and the multi-port valve 104, and (26) analyzing the digested molecules using the analytical device.

[0081] FIG. 8 partially illustrates another example of a method 800 of automatically (or substantially automatically) performing a sixth real-time assay of a sample using the system 100. In this example, the assay is an synthetic product assay and the method 800 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to the first column via the multi-port valve 112 (which is in the first position), (5) capturing the molecules in the sample in the first column, thereby separating the molecules in the sample from a matrix of the sample, which flows out of the first column and to waste via a second vial (one of the receiving vials) fluidly coupled thereto, (6) moving an elution buffer solution from a buffer source to the first column via the needle, the needle seat port, and the multi-port valves 104, 108, and 112, thereby eluting molecules captured by the first column, (7) moving an elution / molecule mixture including the elution buffer solution and the eluted molecules to a second vial (one of the receiving vials), which separates the molecules in the mixture from a remainder of the mixture, thereby forming an affinity purified sample in the second vial, (8) moving a quenching reagent to the second vial via the needle, (9) moving the affinity purified sample and the quenching reagent from the second vial to the first reaction coil (coil A) via the needle, the multi-port valves 104, 108, and the multi-port valve 112 (which is in the third position), (10) incubating the affinity purified sample and the quenching reagent in the first reaction coil, thereby quenching the molecules, (11) moving the quenched molecules from the first reaction coil to a third vial (another one of the receiving vials) fluidly coupled to the first reaction coil, (12) moving the quenched molecules from the third vial to the analytical device via the needle, the needle seat port, and the multi-port valve 104, and (13) analyzing the quenched molecules using the analytical device.

[0082] FIG. 9 illustrates another example of a method 900 of automatically (or substantially automatically) performing a seventh real-time assay of a sample using the system 100. In this example, the assay is a metabolite analysis and the method 900 generally includes: (1) moving (e.g., automatically moving) the needle to obtain (*e.g.,* automatically) a sample from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to the first column (which in this example takes the form of a tri-mode chromatography column) via the multi-port valve 112 (which is in the first position), (5) capturing the molecules of the sample in the first column, thereby separating the molecules of the sample from a matrix of the sample, which flows out of the first column and to waste via a second vial (one of the receiving vials) fluidly coupled thereto, (6) moving both an elution buffer solution (*e.g.*, a low-salt volatile buffer solution) from a buffer source and an organic solvent from one of the intermediate vials to the first column via the needle, the needle seat port, and the multi-port valves 104, 108, and 112, thereby eluting and diluting molecules captured by the first column, (7) moving an elution / molecule mixture including the elution buffer solution, the organic solvent, and the eluted molecules to a second vial (one of the receiving vials), which separates the molecules in the mixture from a remainder of the mixture, (8) moving the molecules from the second vial to the analytical device (which in this example takes the form of a mass spectrometry device such as a HRAM mass spectrometer) via the needle, the needle seat port, and the multi-port valve 104, and (9) analyzing the metabolites in the molecules using the analytical device. It will be appreciated that because the first column in this example is a tri-mode chromatography column, the method 900 advantageously facilitates the analysis of metabolites without using differential labeling or ion-pair reagents. It will also be appreciated that the method 900 can be used to analyze over two-hundred different metabolites, including, for example, amino acids (and very hydrophilic amino acids such as the leucine isomers), vitamins, nucleotide-sugars, nucleoside analogues, keto acids, carbohydrates, amino alcohols, nucleotides, polyamines, and phospholipids.

[0083] FIG. 10 illustrates a schematic diagram of another example of a system 1000 assembled in accordance with the teachings of the present disclosure. The system 1000 illustrated in FIG. 10 is similar to the system 100 in that the system 1000 also generally includes one or more sample vials, a needle, a sample loop, a metering device, a needle seat port, a first column, a second column, one or more buffer sources, one or more sources of change reagents, one or more sources of carrier solution(s), a reaction coil, a heating element thermally coupled to the reaction coil, one or more receiving vials (*e.g.*, one or more flow-through vials), as well as the four multi-port valves 104, 108, 112, 116, the controller, the analytical device, the first pump 120, and the second pump 124 employed in the system of 100 of FIG. 1, and conduit (e.g., stainless steel conduit) extending between each of the components of the system 1000 so as to facilitate fluid communication between components of the system 1000 when desired. However, the system 1000 illustrated in FIG. 10 is different from the system 100 illustrated in FIG. 1 in two main respects. First, unlike the system 100, the system 1000 does not include a second reaction coil. Second, some of the above-described components are arranged differently in the system 1000 than they are arranged in the system 100. As an example, in the system 1000, the analytical device, the first pump 120, and the second pump 124 are fluidly coupled to ports of the multi-port valve 108 instead of ports of the multi-port valve 104 (as they are in the system 100). As another example, in the system 1000, the reaction coil (and the heating element thermally coupled thereto) are fluidly coupled to (and between) ports of the multi-port valve 112 instead of ports of the multi-port valve 116 (as they are in the system 100). Thus, in the system 1000, it is the position of the multi-port valve 112 (and not the position of the multi-port valve 116) that determines whether the multi-port valve 108 is fluidly coupled to the reaction coil (and the heating element).

[0084] Despite these differences, the system 1000 is, like the system 100, a closed system for automatically or substantially automatically preparing a sample of a product containing molecules for analysis and automatically performing an assay of that

sample. By automating (or substantially automating) this process using the system 1000, the assay can be performed in real-time (or substantially in real-time) and on the manufacturing floor (when used in the manufacturing facility), such that the entire process can be performed, and the desired result obtained, in a matter of hours (*e.g.*, 2 to 3 hours), a significant improvement over the few days to a few weeks typically entailed by the conventionally known, manually-operated processes. Moreover, the closed nature of the process that utilizes the system 1000 maintains sterile conditions. Further yet, like the system 100, the system 1000 is a flexible system that can be used to prepare any number of samples (the same or different) for different analyses and can automatically perform a number of different assays on these samples, thereby obviating the need for multiple different systems for preparing different samples for different analyses.

[0085] FIG. 11 illustrates an example of a method 1100 of automatically (or substantially automatically) performing a first real-time assay of a sample using the system 1000. In this example, the assay is a production titer measurement and the method 1100 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample from one of the sample vials and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the analytical device, which in this example takes the form of a liquid chromatography device, via the multi-port valve 104 and the multi-port valve 108, and (4) determining the titer / concentration of the sample using the analytical device.

[0086] FIG. 12 illustrates another example of a method 1200 of automatically (or substantially automatically) performing a second real-time assay of a sample using the system 1000. In this example, the assay is an size-exclusion chromatography (SEC) assay and the method 1200 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample comprising molecules from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to the first column via the multi-port valve 112 (which is in the second position), (5) capturing the molecules in the sample in the first column, thereby separating the molecules in the sample from a matrix of the sample, which flows out of the first column and to waste fluidly coupled thereto, (6) moving an elution buffer solution from a buffer source to the first column via the needle, the needle seat port, and the multi-port valves 104, 108, and 112, thereby eluting molecules captured by the first column, (7) moving an elution / molecule mixture including the elution buffer solution and the eluted molecules to a second vial (one of the receiving vials), which separates the molecules in the mixture from a remainder of the mixture, (8) moving the molecules from the second vial to the analytical device via the needle, the needle seat port, and the multi-port valves 104, 108, and (9) quantifying the level of aggregation in the molecules using the analytical device.

[0087] FIG. 13 illustrates another example of a method 1300 of automatically (or substantially automatically) performing a third real-time assay of a sample using the system 1000. In this example, the assay is an ion-exchange chromatography (IEX) assay and the method 1300 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample (in which the sample comprises molecules) from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to the first column via the multi-port valve 112 (which is in the second position), (5) capturing the molecules in the sample in the first column, thereby separating the molecules in the sample from a matrix of the sample, which flows out of the first column and to waste fluidly coupled thereto, (6) moving an elution buffer solution from a buffer source to the first column via the needle, the needle seat port, and the multi-port valves 104, 108, and 112, thereby eluting molecules captured by the first column, (7) moving an elution / molecule mixture including the elution buffer solution and the eluted molecules to a second vial (one of the receiving vials), which separates the molecules in the mixture from a remainder

of the mixture, thereby forming an affinity purified sample in the second vial, (8) moving the molecules from the second vial to a third vial (one of the intermediate vials) to dilute the affinity purified sample, (9) moving the affinity purified sample from the third vial back to the multi-port valve 108 via the needle, the needle seat port, and the multi-port valve 104, (10) moving the affinity purified sample from the multi-port valve 108 to the second column via the multi-port valve 112 (which has been moved to the third position), (11) applying the affinity purified sample to the second column, thereby further reducing the salt concentration of the sample, (12) moving the further de-salted affinity purified sample from the second column back to the second vial, (13) moving the affinity purified sample from the second vial to the analytical device via the needle, the needle seat port, and the multi-port valves 104, 108, and (14) performing a charge variant assessment using the analytical device (which in this example takes the form of an ion exchange column).

[0088] FIG. 14 illustrates another example of a method 1400 of automatically (or substantially automatically) performing a fourth real-time assay of a sample using the system 1000. In this example, the assay is a MAM assay and the method 1400 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to the first column via the multi-port valve 112 (which is in the second position), (5) capturing the molecules in the sample in the first column, thereby separating the molecules in the sample from a matrix of the sample, which flows out of the first column and to waste fluidly coupled thereto, (6) moving an elution buffer solution from a buffer source to the first column via the needle, the needle seat port, and the multi-port valves 104, 108, and 112, thereby eluting molecules captured by the first column, (7) moving an elution/molecule mixture including the elution buffer solution and the eluted molecules to a second vial (one of the receiving vials), which separates the molecules in the mixture from a remainder of the mixture, thereby forming an affinity purified sample in the second vial, (8) moving, via the needle, the affinity purified sample from the second vial to a third vial (one of the intermediate vials) to dilute the affinity purified sample, (9) moving the diluted affinity purified sample from the third vial back to the second vial via the needle, (10) moving a denaturing reagent and a reducing reagent to the second vial via the needle, (11) moving the affinity purified sample, the denaturing reagent, and the reducing reagent from the second vial to the reaction coil via the needle, the multi-port valves 104, 108, and the multi-port valve 112 (which is in the fourth position), (12) incubating the mixture in the reaction coil, thereby denaturing and reducing the molecules, (13) moving the denatured and reduced molecules from the reaction coil to a fourth vial (another one of the receiving vials) fluidly coupled to the reaction coil, (14) moving an alkylating reagent to the fourth vial via the needle, (15) moving the denatured and reduced molecules and the alkylating reagent from the fourth vial to the reaction coil, via the needle, the multi-port valves 104, 108, and the multi-port valve 112 (which is in the fourth position), (16) incubating the denatured and reduced molecules and the alkylating reagent in the reaction coil, thereby alkylating the denatured and reduced molecules, (17) moving the denatured, reduced, and alkylated molecules from the reaction coil to the fourth vial, (18) moving the denatured, reduced, and alkylated molecules from the fourth vial to the second column via the needle, the needle seat port, the multi-port valves 104, 108, and the multi-port valve 112 (which has been moved to the third position), (19) applying the denatured, reduced, and alkylated molecules to the second column, thereby reducing the salt concentration of the molecules, (20) moving the desalted molecules from the second column back to the second vial, (21) moving an enzyme to the second vial via the needle (either before step (20) or after step (20), (22) moving the desalted molecules and the enzyme from the second vial to the reaction coil via the needle, the multi-port valves 104, 108, and the multi-port valve 112 (which has been moved to the fourth position), (23) incubating the desalted molecules and the enzyme in the reaction coil, thereby digesting the molecules, (24) moving the digested molecules from the reaction coil back

to the fourth vial, (25) moving the digested molecules from the fourth vial to the analytical device via the needle, the needle seat port, and the multi-port valves 104, 108, and (26) analyzing the digested molecules using the analytical device.

[0089] FIG. 15 partially illustrates another example of a method 1500 of automatically performing a fifth real-time assay of a sample using the system 1000. In this example, the assay is a non-chromatographic assay and the method 1500 generally includes: (1) moving (e.g., automatically moving) the needle to obtain a sample from a first vial (one of the sample vials) and to deposit the sample in the sample loop, (2) pushing the sample from the sample loop into the needle seat port, (3) moving the sample from the needle seat port to the multi-port valve 108 via the multi-port valve 104, (4) moving the sample from the multi-port valve 108 to a second vial (one of the receiving vials) via the multi-port valves 108, 112, (5) moving a quenching reagent to the second vial via the needle, (6) moving the sample and the quenching reagent from the second vial to the reaction coil via the needle, the multi-port valves 104, 108, and the multi-port valve 112 (which is in the fourth position), (7) incubating the sample and the quenching reagent in the reaction coil, thereby quenching the molecules in the sample, (8) moving the quenched molecules from the reaction coil back to the second vial or to a third vial (another one of the receiving vials) fluidly coupled to the reaction coil, (9) moving the quenched molecules from the third vial to the analytical device via the needle, the needle seat port, and the multi-port valves 104, 108, and (10) analyzing the quenched molecules using the analytical device.

[0090] FIGS. 16 and 17 illustrate one example of a flow-through vial 1600 that can be employed in the system 100 or the system 1000 to enable continuous flow through the system 100 or the system 1000. As illustrated, the flow-through vial 1600 in this example generally includes a base 1604, a lip 1606 coupled to the base 1604, and a gutter 1608 that is coupled to and extend outwards from both the base 1604 and the lip 1606. In this example, the base 1604 is defined by a first, substantially cylindrical portion 1612 and a second, substantially rectangular portion 1616 coupled to the first, substantially cylindrical portion 1612. The first, substantially cylindrical portion 1612 generally extends from a first end 1620 to a second end 1624 opposite the first end 1620 along a first axis 1628. The first, substantially cylindrical portion 1612 has a substantially cylindrical bore 1632 formed therein between the first end 1620 and the second end 1624. The second, substantially rectangular portion 1616 extends outward from the first, substantially cylindrical portion 1612 along a second axis 1636 that is perpendicular to the first axis 1628. In other examples, however, the flow-through vial components may have different shapes. For example, the gutter 1608 may have a cylindrical or other non-rectangular shape. As another example, the base 1604 can have a cylindrical or substantially cylindrical shape defined only by the substantially cylindrical portion 1612.

[0091] In this example, the lip 1606 is a substantially cylindrical lip that is integrally formed with and extends outward from the first, substantially cylindrical portion 1612 (particularly the first end 1620 thereof) along the first axis 1628. So arranged, the lip 1606 fluidly connects the substantially cylindrical bore 1632 to the gutter 1608. In other examples, however, the lip 1606 can have a different shape, can be fixedly or removably coupled to the first, substantially cylindrical portion 1612 (or another portion), and/or can extend outward from the first, substantially cylindrical portion 1612 along an axis that is angled relative to the first axis 1628. In yet other examples, the flow-through vial 1600 may not include the lip 1606 at all, in which case the gutter 1608 is directly fluidly connected to the substantially cylindrical bore 1632.

[0092] In this example, the gutter 1608 is defined by a substantially rectangular base 1640 and a downspout 1644 that is coupled to the rectangular base 1640. The substantially rectangular base 1640 of the gutter 1608 extends outward from the second, substantially rectangular portion 1616 of the base 1604 and from the lip 1606 along a third axis 1648 that is perpendicular to both the first axis 1628 and the second axis 1636 and is inclined relative to the horizontal (see FIG. 17). The substantially rectangular base 1640 defines a substantially rectangular channel 1652 that fluidly connects the lip 1606 (and in turn the substantially cylindrical bore

1632) to the downspout 1644. The downspout 1644 in this example is cylindrical and extends outward (downward in FIG. 16) from the rectangular base 1640 along a fourth axis 1652 that is parallel to the first axis 1628 and perpendicular to the second axis 1636 and the third axis 1648. In other examples, the gutter 1608 can have a differently-shaped base 1640 (e.g., the base 1640 can have a cylindrical shape), and/or the downspout 1644 can have a different shape. In other examples, the gutter 1608 need not include the downspout 1644.

[0093] The flow-through vial 1600 also includes an inlet port 1660 and an outlet port 1664. The inlet port 1660 is generally arranged in the base 1604 of the flow-through vial so as to receive incoming fluid from the automated sampling system, the multi-port valve 112, the multi-port valve 116, or other components of the system 100 or the system 1000, as desired. As illustrated in FIGS. 16 and 17, the inlet port 1660 in this example formed in the second, substantially rectangular portion 1616 and extends along a fifth axis 1668 that is inclined relative to reach of the first axis 1628, the second axis 1636, the third axis 1648, and the fourth axis 1652. The fifth axis 1668 may, for example, be oriented at an angle of approximately 45 degrees relative to the first axis 1628. Meanwhile, the outlet port 1664 is generally arranged in the gutter 1608 of the flow-through vial so as to direct fluid that has flowed into the base 1604 via the inlet port 1660 and flowed through the substantially cylindrical bore 1632 and the channel 1652 of the gutter 1608 out of the flow-through vial 1600. In this example, the outlet port 1664 is formed in the downspout 1644, though in other examples (e.g., when the flow-through vial 1600 does not include the downspout 1644), the outlet port 1664 can be formed in a different part of the gutter 1608.

[0094] While not illustrated herein, it will be appreciated that conduit (not shown) can be used to fluidly connect the desired component of the system 100 (e.g., the automated sampling system) or the system 1000 to the inlet port 1660. Fluid will in turn flow from that desired component of the system into the flow-through vial 1600 via the inlet port 1660. As the fluid flows through the flow-through vial 1600, the lip 1606 helps to prevent surface tension. In some examples, conduit (not shown) can also be used to fluidly connect the outlet port 1664 to the desired destination for the fluid within the system 100 or the system 1000. As an example, conduit can be used to fluidly connect the outlet port 1664 to the analytical device of the system 100 or the system 1000. In other examples, conduit may not be used to fluidly connect the outlet port 1664 to the desired destination. For example, the needle of the system 100 or the system 1000 can retrieve the fluid directly from the flow-through vial 1600 and deposit the fluid into the desired component of the system 100 (e.g., the sample loop) or the system 1000. As another example (e.g., when it is necessary to clean the flow-through vial 1600), the flow-through vial 1600 can be positioned so that the downspout 1644 is placed directly over the waste, such that the fluid flowing through gutter 1608 is directed to waste. In any event, the flow-through vial 1600 is configured to facilitate continuous flow therethrough, which, for example, obviates the need to periodically change out sample vials (particularly when different samples are being tested).

[0095] FIGS. 18 and 19 illustrate a vial holder 1800 that may be employed in the system 100 or the system 1000 to hold a plurality of the flow-through vials 1600. In this example, the vial holder 1800 is configured to be disposed within or otherwise coupled to the automatic sampling system. In other examples, however, the vial holder 1800 can be coupled to another component of the system 100 or the system 1000. In any event, the vial holder 1800 illustrated in FIGS. 18 and 19 includes a mounting flange 1804 and a plurality of vial receptacles 1808 coupled to the mounting flange 1804. As illustrated in FIG. 19, the mounting flange 1804 can be coupled to a portion of the automatic sampling system so as to couple the via holder 1800 (and the flow-through vials 1600 carried by the vial holder 1800) to the automatic sampling system. Meanwhile, each of the plurality of vial receptacles 1808 is sized to receive one of the flow-through vials 1600. More particularly, as illustrated in FIG. 19, each of the plurality of vial receptacles 1808 is

sized to receive the respective base 1604 of one of the flow-through vials 1600. In turn, the respective gutter 1608 of that flow-through vial 1600 extends outward, away from the via holder 1600.

Therapeutic polypeptides

[0096] Proteins, including those that bind to one or more of the following, can be useful in the disclosed systems and methods. These include CD proteins, including CD3, CD4, CD8, CD19, CD20, CD22, CD30, and CD34; including those that interfere with receptor binding. HER receptor family proteins, including HER2, HER3, HER4, and the EGF receptor. Cell adhesion molecules, for example, LFA-I, Mol, pI50, 95, VLA-4, ICAM-I, VCAM, and alpha v/beta 3 integrin. Growth factors, such as vascular endothelial growth factor ("VEGF"), growth hormone, thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, growth hormone releasing factor, parathyroid hormone, Mullerian-inhibiting substance, human macrophage inflammatory protein (MIP-I -alpha), erythropoietin (EPO), nerve growth factor, such as NGF-beta, platelet-derived growth factor (PDGF), fibroblast growth factors, including, for instance, aFGF and bFGF, epidermal growth factor (EGF), transforming growth factors (TGF), including, among others, TGF- α and TGF-β, including TGF-βl, TGF-β2, TGF-β3, TGF- β4, or TGF- β 5, insulin-like growth factors-I and -II (IGF-I and IGF-II), des(I-3)-IGF-I (brain IGF-I), and osteoinductive factors. Insulins and insulin-related proteins, including insulin, insulin A-chain, insulin B-chain, proinsulin, and insulin-like growth factor binding proteins. Coagulation and coagulation-related proteins, such as, among others, factor VIII, tissue factor, von Willebrands factor, protein C, alpha-1-antitrypsin, plasminogen activators, such as urokinase and tissue plasminogen activator ("t-PA"), bombazine, thrombin, and thrombopoietin; (vii) other blood and serum proteins, including but not limited to albumin, IgE, and blood group antigens. Colony stimulating factors and receptors thereof, including the following, among others, M-CSF, GM-CSF, and G-CSF, and receptors thereof, such as CSF-1 receptor (c-fms). Receptors and receptor-associated proteins, including, for example, flk2/flt3 receptor, obesity (OB) receptor, LDL receptor, growth hormone receptors, thrombopoietin receptors ("TPO-R," "c-mpl"), glucagon receptors, interleukin receptors, interferon receptors, T-cell receptors, stem cell factor receptors, such as c-Kit, and other receptors. Receptor ligands, including, for example, OX40L, the ligand for the OX40 receptor. Neurotrophic factors, including bone-derived neurotrophic factor (BDNF) and neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6). Relaxin A-chain, relaxin B-chain, and prorelaxin; interferons and interferon receptors, including for example, interferon-α, -β, and -γ, and their receptors. Interleukins and interleukin receptors, including IL-I to IL-33 and IL-I to IL-33 receptors, such as the IL-8 receptor, among others. Viral antigens, including an AIDS envelope viral antigen. Lipoproteins, calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha and -beta, enkephalinase, RANTES (regulated on activation normally T-cell expressed and secreted), mouse gonadotropin-associated peptide, DNAse, inhibin, and activin. Integrin, protein A or D, rheumatoid factors, immunotoxins, bone morphogenetic protein (BMP), superoxide dismutase, surface membrane proteins, decay accelerating factor (DAF), AIDS envelope, transport proteins, homing receptors, addressins, regulatory proteins, immunoadhesins, antibodies. Myostatins, TALL proteins, including TALL-I, amyloid proteins, including but not limited to amyloid-beta proteins, thymic stromal lymphopoietins ("TSLP"), RANK ligand ("OPGL"), c-kit, TNF receptors, including TNF Receptor Type 1, TRAIL-R2, angiopoietins, and biologically active fragments or analogs or variants of any of the foregoing.

[0097] Exemplary polypeptides and antibodies include Activase® (Alteplase); alirocumab, Aranesp® (Darbepoetin-alfa), Epogen® (Epoetin alfa, or erythropoietin); Avonex® (Interferon β-Ia); Bexxar® (Tositumomab); Betaseron® (Interferon-β); bococizumab (anti-PCSK9 monoclonal antibody designated as L1L3, see US8080243); Campath® (Alemtuzumab); Dynepo® (Epoetin delta); Velcade® (bortezomib); MLN0002 (anti-α4β7 mAb); MLN1202 (anti-CCR2 chemokine receptor mAb); Enbrel® (etanercept); Eprex® (Epoetin alfa); Erbitux® (Cetuximab); evolocumab; Genotropin® (Somatropin); Herceptin® (Trastuzumab);

Humatrope® (somatropin [rDNA origin] for injection); Humira® (Adalimumab); Infergen® (Interferon Alfacon-1); Natrecor® (nesiritide); Kineret® (Anakinra), Leukine® (Sargamostim); LymphoCide® (Epratuzumab); BenlystaTM (Belimumab); Metalyse® (Tenecteplase); Mircera® (methoxy polyethylene glycol-epoetin beta); Mylotarg® (Gemtuzumab ozogamicin); Raptiva® (efalizumab); Cimzia® (certolizumab pegol); SolirisTM (Eculizumab); Pexelizumab (Anti-C5 Complement); MEDI-524 (Numax®); Lucentis® (Ranibizumab); Edrecolomab (,Panorex®); Trabio® (lerdelimumab); TheraCim hR3 (Nimotuzumab); Omnitarg (Pertuzumab, 2C4); Osidem® (IDM-I); OvaRex® (B43.13); Nuvion® (visilizumab); Cantuzumab mertansine (huC242-DMI); NeoRecormon® (Epoetin beta); Neumega® (Oprelvekin); Neulasta® (Pegylated filgastrim, pegylated G-CSF, pegylated hu-Met-G-CSF); Neupogen® (Filgrastim); Orthoclone OKT3® (Muromonab-CD3), Procrit® (Epoetin alfa); Remicade® (Infliximab), Reopro® (Abciximab), Actemra® (anti-IL6 Receptor mAb), Avastin® (Bevacizumab), HuMax-CD4 (zanolimumab), Rituxan® (Rituximab); Tarceva® (Erlotinib); Roferon-A®-(Interferon alfa-2a); Simulect® (Basiliximab); StelaraTM (Ustekinumab); Prexige® (lumiracoxib); Synagis® (Palivizumab); 146B7-CHO (anti-IL15 antibody, see US7153507), Tysabri® (Natalizumab); Valortim® (MDX-1303, anti-B. anthracis Protective Antigen mAb); ABthraxTM; Vectibix® (Panitumumab); Xolair® (Omalizumab), ETI211 (anti-MRSA mAb), IL-I Trap (the Fc portion of human IgGI and the extracellular domains of both IL-I receptor components (the Type I receptor and receptor accessory protein)), VEGF Trap (Ig domains of VEGFRI fused to IgGI Fc), Zenapax® (Daclizumab); Zenapax® (Daclizumab), Zevalin® (Ibritumomab tiuxetan), Zetia (ezetimibe), Atacicept (TACI-Ig), anti-α4β7 mAb (vedolizumab); galiximab (anti-CD80 monoclonal antibody), anti-CD23 mAb (lumiliximab); BR2-Fc (huBR3 / huFc fusion protein, soluble BAFF antagonist); SimponiTM (Golimumab); Mapatumumab (human anti-TRAIL Receptor-1 mAb); Ocrelizumab (anti-CD20 human mAb); HuMax-EGFR (zalutumumab); M200 (Volociximab, anti-α5β1 integrin mAb); MDX-010 (Ipilimumab, anti-CTLA-4 mAb and VEGFR-I (IMC-18F1); anti-BR3 mAb; anti-C. difficile Toxin A and Toxin B C mAbs MDX-066 (CDA-I) and MDX-1388); anti-CD22 dsFv-PE38 conjugates (CAT-3888 and CAT-8015); anti-CD25 mAb (HuMax-TAC); anti-TSLP antibodies; anti-TSLP receptor antibody (US8101182); anti-TSLP antibody designated as A5 (US7982016); (anti-CD3 mAb (NI-0401); Adecatumumab (MT201, anti-EpCAM-CD326 mAb); MDX-060, SGN-30, SGN-35 (anti-CD30 mAbs); MDX-1333 (anti- IFNAR); HuMax CD38 (anti-CD38 mAb); anti-CD40L mAb; anti-Cripto mAb; anti-CTGF Idiopathic Pulmonary Fibrosis Phase I Fibrogen (FG-3019); anti-CTLA4 mAb; anti-eotaxinl mAb (CAT-213); anti-FGF8 mAb; anti-ganglioside GD2 mAb; anti-sclerostin antibodies (see, US8715663 or US7592429) anti-sclerostin antibody designated as Ab-5 (US8715663 or US7592429); anti-ganglioside GM2 mAb; anti-GDF-8 human mAb (MYO-029); anti-GM-CSF Receptor mAb (CAM-3001); anti-HepC mAb (HuMax HepC); MEDI-545, MDX-1103 (anti-IFNα mAb); anti-IGFIR mAb; anti-IGF-IR mAb (HuMax-Inflam); anti-IL12/IL23p40 mAb (Briakinumab); anti-IL-23p19 mAb (LY2525623); anti-IL13 mAb (CAT-354); anti-IL-17 mAb (AIN457); anti-IL2Ra mAb (HuMax-TAC); anti-IL5 Receptor mAb; anti-integrin receptors mAb (MDX-OI8, CNTO 95); anti-IPIO Ulcerative Colitis mAb (MDX- 1100); anti-LLY antibody; BMS-66513; anti-Mannose Receptor/hCGβ mAb (MDX-1307); anti-mesothelin dsFv-PE38 conjugate (CAT-5001); anti-PDImAb (MDX-1 106 (ONO- 4538)); anti-PDGFRα antibody (IMC-3G3); anti-TGFβ mAb (GC-1008); anti-TRAIL Receptor-2 human mAb (HGS-ETR2); anti-TWEAK mAb; anti-VEGFR/Flt-1 mAb; anti- ZP3 mAb (HuMax-ZP3); and an amyloid-beta monoclonal antibody comprising sequences, SEQ ID NO:8 and SEQ ID NO:6 (US7906625).

[0098] Examples of antibodies suitable for the methods and pharmaceutical formulations include the antibodies shown in Table 1. Other examples of suitable antibodies include infliximab, bevacizumab, cetuximab, ranibizumab, palivizumab, abagovomab, abciximab, actoxumab, adalimumab, afelimomab, afutuzumab, alacizumab, alacizumab pegol, ald518, alemtuzumab, alirocumab, altumomab, amatuximab, anatumomab mafenatox, anrukinzumab, apolizumab, arcitumomab, aselizumab, altinumab, atlizumab, atorolimiumab, tocilizumab, bapineuzumab, basiliximab, bavituximab, bectumomab, belimumab, benralizumab, bertilimumab, besilesomab, bevacizumab, bezlotoxumab, biciromab, bivatuzumab, bivatuzumab mertansine, blinatumomab, blosozumab,

brentuximab vedotin, briakinumab, brodalumab, canakinumab, cantuzumab mertansine, cantuzumab mertansine, caplacizumab, capromab pendetide, carlumab, catumaxomab, cc49, cedelizumab, certolizumab pegol, cetuximab, citatuzumab bogatox, cixutumumab, clazakizumab, clenoliximab, clivatuzumab tetraxetan, conatumumab, crenezumab, cr6261, dacetuzumab, daclizumab, dalotuzumab, daratumumab, demcizumab, denosumab, detumomab, dorlimomab aritox, drozitumab, duligotumab, dupilumab, ecromeximab, eculizumab, edobacomab, edrecolomab, efalizumab, efungumab, elotuzumab, elsilimomab, enavatuzumab, enlimomab pegol, enokizumab, enoticumab, ensituximab, epitumomab cituxetan, epratuzumab, erenumab, erlizumab, ertumaxomab, etaracizumab, etrolizumab, evolocumab, exbivirumab, fanolesomab, faralimomab, farletuzumab, fasinumab, fbta05, felvizumab, fezakinumab, ficlatuzumab, figitumumab, flanvotumab, fontolizumab, foralumab, foravirumab, fresolimumab, fulranumab, futuximab, galiximab, ganitumab, gantenerumab, gavilimomab, gemtuzumab ozogamicin, gevokizumab, girentuximab, glembatumumab vedotin, golimumab, gomiliximab, gs6624, ibalizumab, ibritumomab tiuxetan, icrucumab, igovomab, imciromab, imgatuzumab, inclacumab, indatuximab ravtansine, infliximab, intetumumab, inolimomab, inotuzumab ozogamicin, ipilimumab, iratumumab, itolizumab, ixekizumab, keliximab, labetuzumab, lebrikizumab, lemalesomab, lerdelimumab, lexatumumab, libivirumab, ligelizumab, lintuzumab, lirilumab, lorvotuzumab mertansine, lucatumumab, lumiliximab, mapatumumab, maslimomab, mavrilimumab, matuzumab, mepolizumab, metelimumab, milatuzumab, minretumomab, mitumomab, mogamulizumab, morolimumab, motavizumab, moxetumomab pasudotox, muromonab-cd3, nacolomab tafenatox, namilumab, naptumomab estafenatox, narnatumab, natalizumab, nebacumab, necitumumab, nerelimomab, nesvacumab, nimotuzumab, nivolumab, nofetumomab merpentan, ocaratuzumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, olokizumab, omalizumab, onartuzumab, oportuzumab monatox, oregovomab, orticumab, otelixizumab, oxelumab, ozanezumab, ozoralizumab, pagibaximab, palivizumab, panitumumab, panobacumab, parsatuzumab, pascolizumab, pateclizumab, patritumab, pemtumomab, perakizumab, pertuzumab, pexelizumab, pidilizumab, pintumomab, placulumab, ponezumab, priliximab, pritumumab, PRO 140, quilizumab, racotumomab, radretumab, rafivirumab, ramucirumab, ranibizumab, raxibacumab, regavirumab, reslizumab, rilotumumab, rituximab, robatumumab, roledumab, romosozumab, rontalizumab, rovelizumab, ruplizumab, samalizumab, sarilumab, satumomab pendetide, secukinumab, sevirumab, sibrotuzumab, sifalimumab, siltuximab, simtuzumab, siplizumab, sirukumab, solanezumab, solitomab, sonepcizumab, sontuzumab, stamulumab, sulesomab, suvizumab, tabalumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tanezumab, taplitumomab paptox, tefibazumab, telimomab aritox, tenatumomab, tefibazumab, teneliximab, teplizumab, teprotumumab, tezepelumab, TGN1412, tremelimumab, ticilimumab, tildrakizumab, tigatuzumab, TNX-650, tocilizumab, toralizumab, tositumomab, tralokinumab, trastuzumab, TRBS07, tregalizumab, tucotuzumab celmoleukin, tuvirumab, ublituximab, urelumab, urtoxazumab, ustekinumab, vapaliximab, vatelizumab, vedolizumab, veltuzumab, vepalimomab, vesencumab, visilizumab, volociximab, vorsetuzumab mafodotin, votumumab, zalutumumab, zanolimumab, zatuximab, ziralimumab, zolimomab aritox.

[0099] Antibodies also include adalimumab, bevacizumab, blinatumomab, cetuximab, conatumumab, denosumab, eculizumab, erenumab, evolocumab, infliximab, natalizumab, panitumumab, rilotumumab, rituximab, romosozumab, tezepelumab, and trastuzumab, and antibodies selected from Table 1.

Target (informal name)	Conc. (mg/ml)	Viscosity (cP)	HC Type (including allotypes)	LC Type	pI	LC SEQ ID NO	HC SEQ ID NO
anti-amyloid	142.2	5.0	IgG1 (f) (R;EM)	Kappa	9.0	1	2
GMCSF (247)	139.7	5.6	IgG2	Kappa	8.7	3	4
CGRPR	136.6	6.3	IgG2	Lambda	8.6	5	6
RANKL	152.7	6.6	IgG2	Kappa	8.6	7	8
Sclerostin (27H6)	145.0	6.7	IgG2	Kappa	6.6	9	10
IL-1R1	153.9	6.7	IgG2	Kappa	7.4	11	12
Myostatin	141.0	6.8	IgG1 (z) (K;EM)	Kappa	8.7	13	14
B7RP1	137.5	7.7	IgG2	Kappa	7.7	15	16
Amyloid	140.6	8.2	IgG1 (za) (K;DL)	Kappa	8.7	17	18
GMCSF (3.112)	156.0	8.2	IgG2	Kappa	8.8	19	20
CGRP (32H7)	159.5	8.3	IgG2	Kappa	8.7	21	22
CGRP (3B6.2)	161.1	8.4	IgG2	Lambda	8.6	23	24
PCSK9 (8A3.1)	150.0	9.1	IgG2	Kappa	6.7	25	26
PCSK9 (492)	150.0	9.2	IgG2	Kappa	6.9	27	28
CGRP	155.2	9.6	IgG2	Lambda	8.8	29	30
Hepcidin	147.1	9.9	IgG2	Lambda	7.3	31	32
TNFR p55)	157.0	10.0	IgG2	Kappa	8.2	33	34
OX40L	144.5	10.0	IgG2	Kappa	8.7	35	36
HGF	155.8	10.6	IgG2	Kappa	8.1	37	38
GMCSF	162.5	11.0	IgG2	Kappa	8.1	39	40
Glucagon R	146.0	12.1	IgG2	Kappa	8.4	41	42
GMCSF (4.381)	144.5	12.1	IgG2	Kappa	8.4	43	44
Sclerostin (13F3)	155.0	12.1	IgG2	Kappa	7.8	45	46
CD-22	143.7	12.2	IgG1 (f) (R;EM)	Kappa	8.8	47	48
INFgR	154.2	12.2	IgG1 (za) (K;DL)	Kappa	8.8	49	50
Ang2	151.5	12.4	IgG2	Kappa	7.4	51	52
TRAILR2	158.3	12.5	IgG1 (f) (R;EM)	Kappa	8.7	53	54
EGFR	141.7	14.0	IgG2	Kappa	6.8	55	56
IL-4R	145.8	15.2	IgG2	Kappa	8.6	57	58
IL-15	149.0	16.3	IgG1 (f) (R;EM)	Kappa	8.8	59	60
IGF1R	159.2	17.3	IgG1 (za) (K;DL)	Kappa	8.6	61	62
IL-17R	150.9	19.1	IgG2	Kappa	8.6	63	64
Dkk1 (6.37.5)	159.4	19.6	IgG2	Kappa	8.2	65	66
Sclerostin	134.8	20.9	IgG2	Kappa	7.4	67	68
TSLP	134.2	21.4	IgG2	Lambda	7.2	69	70
Dkk1 (11H10)	145.3	22.5	IgG2	Kappa	8.2	71	72

Target (informal name)	Conc. (mg/ml)	Viscosity (cP)	HC Type (including allotypes)	LC Type	pI	LC SEQ ID NO	HC SEQ ID NO
PCSK9	145.2	22.8	IgG2	Lambda	8.1	73	74
GIPR (2G10.006)	150.0	23.0	IgG1 (z) (K;EM)	Kappa	8.1	75	76
Activin	133.9	29.4	IgG2	Lambda	7.0	77	78
Sclerostin (2B8)	150.0	30.0	IgG2	Lambda	6.7	79	80
Sclerostin	141.4	30.4	IgG2	Kappa	6.8	81	82
c-fms	146.9	32.1	IgG2	Kappa	6.6	83	84
α4β7	154.9	32.7	IgG2	Kappa	6.5	85	86

* An exemplary concentration suitable for patient administration; ^HC – antibody heavy chain; LC – antibody light chain.

[00100] Based on the foregoing description, it should be appreciated that the devices, systems, and methods described herein facilitate the automatic (or substantially automatic) preparation of a sample of a product containing molecules for analysis and the automatic (or substantially automatic) performance of an assay of that sample. As used herein "automatic" has its ordinary and customary meaning as would be understood by one of ordinary skill in the art in view of this disclosure. It refers to performing a process without human intervention during the process. An automatic process may be performed by one or more machines. As used herein "substantially automatic" has its ordinary and customary meaning as would be understood by one of ordinary skill in the art in view of this disclosure. It refers to performing a process in which more active steps are performed without human intervention than with human intervention. If additional numerical information is of interest, a process in which at least 51%, 60%, 70%, 80%, or 90% of the process steps are performed without human intervention may be considered "substantially automatic." For example, a process that runs for a day and involves 0.5 – 1 hours of "staff hand time" during the day may be considered to be substantially automatic. As used herein "continuous" has its ordinary and customary meaning as would be understood by one of ordinary skill in the art in view of this disclosure. It refers to performing at least two cycles of a process without interruption. The at least two cycles may be serial (e.g., performed subsequent to each other), or may overlap. Thus, the preparation and analysis can be performed substantially in-real time. In other words, the entire process can be performed much more quickly than presently allowed by conventional processes.

[00101] Moreover, the Applicant has discovered that assays performed using the systems and methods described herein yield results that are comparable to results yielded by assays performed using conventional techniques, thereby validating the effectiveness of the systems and methods described herein. In particular, the Applicant performed three different assays – a size-exclusion chromatography (SEC) assay, a cation-exchange chromatography (CEX) assay, and a reduced capillary electrophoresis (rCE) assay – with each of 9 different molecules – molecules mAb1, mAb2, mAb3, BiTE1, BiTE2, Bi-specific 1, Fusion, Bi-specific 2, and xmAb – using both the systems and methods described herein and a conventional technique. While there are a number of conventional techniques for sample affinity purification (e.g., PhyNexus PhyTips, GE PreDictor plates, Hamilton Leap autosampler and Tecan-Atoll), Tecan-Atoll purification was used as the conventional technique in this example. It is believed to be representative of conventional techniques. As illustrated in FIG. 20, the Applicant discovered that the results of 25 of the 27 different assays were comparable to the results of the same 27 assays performed using the conventional technique, as demonstrated by the 25 different check marks. The remaining two assays could not be compared because results from the conventional technique were not available. As used herein, "comparable" has its ordinary and customary meaning as understood by one of ordinary skill in the art in view of this disclosure. For example, it has been observed herein that the main peaks yielded by the different assays performed by the disclosed

systems and methods were within 5% of the main peaks yielded by the different assays performed using the conventional technique, which would be understood to yield "comparable" results to the conventional technique.

[00102] FIGS. 21 and 22 graphically illustrate some of the results supporting the Applicant's findings. In particular, FIGS. 21 and 22 graphically illustrate the results of the rCE assay performed with the Bi-specific 1 molecule using the systems and methods described herein and using the conventional technique, respectively. As illustrated, the rCE assay performed with the Bi-specific 1 molecule using the systems and methods described herein produced a Peak A value equal to 15.450 and a Peak B value equal to 19.658, whereas the rCE assay performed with the Bi-specific 1 molecule using the conventional technique produced a Peak A value equal to 15.183 and a Peak B value equal to 19.458.

[00103] Preferred embodiments of this disclosure are described herein, including the best mode or modes known to the inventors for carrying out the disclosure. Although numerous examples are shown and described herein, those of skill in the art will readily understand that details of the various embodiments need not be mutually exclusive. Instead, those of skill in the art upon reading the teachings herein should be able to combine one or more features of one embodiment with one or more features of the remaining embodiments. Further, it also should be understood that the illustrated embodiments are exemplary only and should not be taken as limiting the scope of the disclosure. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the aspects of the exemplary embodiment or embodiments of the disclosure, and do not pose a limitation on the scope of the disclosure. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the disclosure.

CLAIMS

1. A method comprising the steps of:

(a) moving a sample comprising molecules from a first vial to a sample loop;

(b) moving a volume of the sample from the sample loop to a first multi-port valve;

(c) moving the volume of the sample from the first multi-port valve to a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve;

(d) when the second multi-port valve is in a first position, moving the volume of the sample from the second multi-port valve to a capture column;

(e) capturing the molecules in the sample in the capture column, thereby separating the molecules in the sample from a matrix of the sample;

(f) moving an elution buffer solution from a buffer source to the capture column, thereby eluting molecules captured by the capture column and moving an elution/molecule mixture comprising the elution buffer solution and the eluted molecules to a second vial arranged downstream of the capture column, the second vial comprising a flow-through vial; and

(g) moving the molecules in the second vial to an analytical device for analysis of the molecules.

2. The method of claim 1, further comprising, after (f) and before (g), diluting the eluted molecules in the second vial to reduce a salt concentration of the molecules.

3. The method of claim 1 or 2, further comprising, after (f) and before (g):

moving the second multi-port valve to a second position different from the first position; and

when the second multi-port valve is in the second position, moving the eluted molecules to a desalting column via the first multi-port valve and the second multi-port valve, and applying the eluted molecules to the desalting column, thereby reducing the salt concentration.

4. The method of any one of the preceding claims, further comprising, after (f) and before (g),

adding a quenching reagent to the eluted molecules in the second vial;

moving the quenching reagent and the eluted molecules from the second vial to a reaction coil;

incubating the eluted molecules with the quenching reagent in the reaction coil, thereby quenching the molecules; and

moving the quenched molecules from the reaction coil to the second vial.

5. The method of claim 1, wherein (g) comprises:

adding a denaturing reagent and a reducing reagent to the second vial;

moving the eluted molecules, the denaturing reagent, and the reducing reagent from the second vial to a first reaction coil via the first multi-port valve;

incubating the eluted molecules with the denaturing reagent, and the reducing reagent in the reaction coil, resulting in denatured and reduced molecules;

moving the denatured and reduced molecules to a third vial, the third vial also comprising a flow-through vial; and

moving the denatured and reduced molecules in the third vial to the analytical device.

6. The method of claim 5, further comprising, prior to moving the denatured and reduced molecules in the third vial to the analytical device:

adding an alkylating reagent to the third vial;

moving the denatured and reduced molecules and the alkylating reagent from the third vial to the first reaction coil via the first multi-port valve;

incubating the denatured and reduced molecules with the alkylating reagent in the first reaction coil, thereby alkylating the denatured and reduced molecules;

moving the denatured, reduced, and alkylated molecules from the first reaction coil to the second vial;

moving the denatured, reduced, and alkylated molecules to a desalting column via the first multi-port valve and the second multi-port valve;

applying the denatured, reduced, and alkylated molecules to the desalting column, thereby resulting in desalted molecules;

moving the desalted molecules to the second vial;

combining the desalted molecules with an enzyme;

moving the desalted molecules and the enzyme to a second reaction coil;

incubating the desalted molecules and the enzyme in the second reaction coil, thereby digesting the molecules;

moving the digested molecules to the third vial; and

moving the digested molecules from the third vial to the analytical device for analysis of the digested molecules.

7. The method of claim 5 or 6, wherein the incubating comprises maintaining the first reaction coil at a first predetermined incubation temperature and/or maintaining the second reaction coil at a second predetermined incubation temperature.

8. The method of any one of the preceding claims, wherein (a) comprises automatically moving, with a needle, the sample from the first vial to the sample loop.

9. The method of any one of the preceding claims, further comprising, before (a), automatically depositing the sample in the first vial.

10. The method of any one of claims 1 to 8, further comprising, before (a), manually depositing the sample in the first vial.

11. The method of any one of the preceding claims, wherein one or more of (a) through (h) are performed automatically using a controller.

12. The method of any one of the preceding claims, wherein (a) through (h) are performed in a closed system.

13. The method of any one of claims 1 to 12, wherein the molecules comprise polypeptides, wherein the capture column comprises a polypeptide binding column, and wherein (e) comprises binding the polypeptides in the sample to the polypeptide binding column, thereby separating the polypeptides in the sample from the matrix of the sample.

14. The method of any one of claims 1 to 12, wherein the molecules comprise small molecules, and wherein the capture column comprises a reverse phase column.

15. The method of any one of claims 1, 2, and 8 to 12, wherein the molecules comprise metabolites.

16. The method of any one of claims 1, 2, 8 to 12, and 15, wherein the capture column comprises a tri-mode chromatography column.

17. The method of any one of claims 1, 2, 8 to 12, 15, and 16, wherein the elution buffer solution comprises a volatile buffer solution.

18. The method of any one of claims 1, 2, 8 to 12, and 15 to 17, wherein the analytical device comprises a mass spectrometer.

19. A method comprising the steps of:

(a) moving a sample comprising polypeptides from a first vial to a sample loop;

(b) moving a volume of the sample from the sample loop to a first multi-port valve;

(c) moving the volume of the sample from the first multi-port valve to a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve;

(d) when the second multi-port valve is in a first position, moving the volume of the sample from the second multi-port valve to a polypeptide binding column;

(e) capturing the polypeptides in the sample in the polypeptide binding column, thereby separating the polypeptides in the sample from a matrix of the sample;

(f) moving an elution buffer solution from a buffer source to the polypeptide binding column, thereby eluting polypeptides captured by the polypeptide binding column and moving an elution/molecule mixture comprising the elution buffer solution and the eluted polypeptides to a second vial arranged downstream of the polypeptide binding column, the second vial comprising a flow-through vial; and

(g) moving the polypeptides in the second vial to an analytical device for analysis of the polypeptides.

20. The method of claim 19, further comprising, after (f) and before (g), diluting the eluted polypeptides in the second vial to reduce a salt concentration of the eluted polypeptides.

21. The method of claim 19 or 20, further comprising, after (f) and before (g):

moving the second multi-port valve to a second position different from the first position; and

when the second multi-port valve is in the second position, moving the eluted polypeptides to a desalting column via the first multi-port valve and the second multi-port valve, and applying the eluted polypeptides to the desalting column, thereby reducing the salt concentration.

22. The method of any one of claims 19 to 21, further comprising, after (f) and before (g),

adding a quenching reagent to the eluted polypeptides in the second vial;

moving the quenching reagent and the eluted polypeptides from the second vial to a reaction coil;

incubating the eluted polypeptides with the quenching reagent in the reaction coil, thereby quenching the polypeptides; and

moving the quenched polypeptides from the reaction coil to the second vial.

23. The method of claim 19, wherein (g) comprises:

adding a denaturing reagent and a reducing reagent to the second vial;

moving the eluted polypeptides, the denaturing reagent, and the reducing reagent from the second vial to a first reaction coil via the first multi-port valve;

incubating the eluted polypeptides with the denaturing reagent, and the reducing reagent in the reaction coil, resulting in denatured and reduced polypeptides;

moving the denatured and reduced polypeptides to a third vial, the third vial also comprising a flow-through vial; and

moving the denatured and reduced polypeptides in the third vial to the analytical device.

24. The method of claim 23, further comprising, prior to moving the denatured and reduced polypeptides in the third vial to the analytical device:

adding an alkylating reagent to the third vial;

moving the denatured and reduced polypeptides and the alkylating reagent from the third vial to the first reaction coil via the first multi-port valve;

incubating the denatured and reduced polypeptides with the alkylating reagent in the first reaction coil, thereby alkylating the denatured and reduced polypeptides;

moving the denatured, reduced, and alkylated polypeptides from the first reaction coil to the second vial;

moving the denatured, reduced, and alkylated polypeptides to a desalting column via the first multi-port valve and the second multi-port valve;

applying the denatured, reduced, and alkylated polypeptides to the desalting column, thereby resulting in desalted polypeptides;

moving the desalted polypeptides to the second vial;

adding an enzyme to the desalted polypeptides;

moving the desalted polypeptides and the enzyme to a second reaction coil;

incubating the desalted polypeptides and the enzyme in the second reaction coil, thereby digesting the polypeptides; and

moving the digested polypeptides to the third vial,

whereby the digested polypeptides from the third vial are ready to be moved to the analytical device for analysis of the digested polypeptides.

25. The method of claim 23 or 24, further comprising maintaining the first reaction coil at a first predetermined incubation temperature and/or maintaining the second reaction coil at a second predetermined incubation temperature.

26. The method of any one of claims 19 to 25, wherein (a) comprises automatically moving, with a needle, the sample from the first vial to the sample loop.

27. The method of any one of claims 19 to 26, further comprising, before (a), automatically depositing the sample in the first vial.

28. The method of any one of claims 19 to 26, further comprising, before (a), manually depositing the sample in the first vial.

29. The method of any one of claims 19 to 28, wherein one or more of (a) through (h) are performed automatically using a controller.

30. The method of any one of claims 19 to 29, wherein (a) through (h) are performed in a closed system.

31. The method of claim 19, wherein (e) comprises pre-concentrating the polypeptides in the sample.

32. A method comprising the steps of:

(a) moving a sample comprising polypeptides from a first vial to a sample loop;

(b) moving a volume of the sample from the sample loop to a first multi-port valve;

(c) moving the volume of the sample from the first multi-port valve to a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve;

(d) moving the volume of the sample from the second multi-port valve to a polypeptide binding column;

(e) binding the polypeptides in the volume of the sample to the polypeptide binding column, thereby separating the polypeptides in the sample from a matrix of the sample;

(f) moving glycosidases to the polypeptide-binding column via the second multi-port valve to release glycans from the bound polypeptides;

(g) moving the released glycans out of the polypeptide-binding column and to a second vial arranged downstream of the polypeptide-binding column, the second vial comprising a flow-through vial;

(h) mixing the released glycans with a glycan-labeling reagent in the second vial;

(i) moving the mixture of the released glycans and the glycan-labeling reagent to a reaction coil via the first multi-port valve;

(j) incubating the mixture of the released glycans and the glycan-labeling reagent in the reaction coil, thereby labeling the glycans;

(k) moving the mixture from the reaction coil to a third vial, the third vial also comprising a flow-through vial; and

(l) moving the labeled glycans in the third vial to an analytical device for analysis of the labeled glycans.

33. The method of claim 32, wherein (h) comprises fluorescently labeling the glycans with the glycan-labeling reagent.

34. The method of claim 32 or 33, further comprising maintaining the reaction coil at a pre-determined incubation temperature.

35. The method of claim 34, wherein the pre-determined temperature comprises approximately 80 degrees Celsius.

36. A method comprising the steps of:

(a) moving a sample comprising polypeptides from a first vial to a sample loop;

(b) moving a volume of the sample from the sample loop to a first multi-port valve;

(c) moving the volume of the sample from the first multi-port valve to a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve;

(d) moving the volume of the sample from the second multi-port valve to a polypeptide binding column;

(e) binding the polypeptides in the volume of the sample to the polypeptide binding column, thereby separating the polypeptides in the sample from a matrix of the sample;

(f) moving glycosidases to the polypeptide-binding column via the second multi-port valve to release glycans from the bound polypeptides;

(g) moving the released glycans out of the polypeptide-binding column and to a second vial arranged downstream of the polypeptide-binding column, the second vial being a flow-through vial;

(h) mixing the released glycans with a glycan-labeling reagent in the second vial;

(i) moving the mixture of the released glycans and the glycan-labeling reagent to a reaction coil via the first multi-port valve;

(j) incubating the mixture of the released glycans and the glycan-labeling reagent in the reaction coil, thereby labeling the glycans;

(k) moving the mixture from the reaction coil to a third vial, the third vial also being a flow-through vial; and

(l) moving the labeled glycans in the third vial to an analytical device for analysis of the labeled glycans.

37. The method of claim 36, wherein (h) comprises fluorescently labeling the glycans with the glycan-labeling reagent.

38. The method of claim 36 or 37, further maintaining the reaction coil at a pre-determined incubation temperature.

39. The method of claim 36, wherein the pre-determined temperature comprises approximately 80 degrees Celsius.

40. A method comprising the steps of:

(a) moving a sample comprising metabolites from a first vial to a sample loop;

(b) moving a volume of the sample from the sample loop to a first multi-port valve;

(c) moving the volume of the sample from the first multi-port valve to a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve;

(d) when the second multi-port valve is in a first position, moving the volume of the sample from the second multi-port valve to a tri-mode chromatography column;

(e) capturing the metabolites in the sample in the tri-mode chromatography column, thereby separating the metabolites in the sample from a matrix of the sample;

(f) moving an elution buffer solution from a buffer source to the tri-mode chromatography column, thereby eluting metabolites captured by the tri-mode chromatography column and moving an elution/molecule mixture comprising the elution buffer solution and the eluted metabolites to a second vial arranged downstream of the tri-mode chromatography column, the second vial comprising a flow-through vial; and

(g) moving the metabolites in the second vial to an analytical device for analysis of the molecules.

41. The method of claim 40, wherein the elution buffer solution comprises a volatile buffer solution.

42. The method of claim 40 or 41, wherein the analytical device comprises a mass spectrometer

43. The method of any one of claims 40 to 42, wherein (a) comprises automatically moving, with a needle, the sample from the first vial to the sample loop.

44. The method of any one of claims 40 to 43, further comprising, before (a), automatically depositing the sample in the first vial.

45. The method of any one of claims 40 to 44, wherein one or more of (a) through (g) are performed automatically using a controller.

46. The method of any one of claims 40 to 45, wherein (a) through (g) are performed in a closed system.

47. The method of any one of claims 1 to 46, wherein the flow-through vial comprises a base, a gutter coupled to and extending outwardly from the base, an inlet port formed in the base, and an outlet port formed in the gutter.

48. The method of claim 47, wherein the flow-through vial further comprises a lip arranged between the base and the gutter, the lip configured to reduce surface tension of fluid flowing through the flow-through vial.

49. The method of claim 47 or 48, wherein the inlet port extends along an inlet axis and the outlet port extends along an outlet axis, the outlet axis being inclined relative to the inlet axis.

50. The method of claim 49, wherein the base extends along a longitudinal axis that is inclined relative to each of the inlet axis and the outlet axis.

51. The method of any one of claims 47 to 50, wherein the gutter comprises a downspout, and wherein the outlet port is formed in the downspout.

52. A closed system, comprising:

a first vial adapted to contain a sample comprising molecules;

one or more change agents;

a sample loop adapted to receive the sample from the first vial;

a first multi-port valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a first port of the first multi-port valve;

a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve;

a capture column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a first position, the capture column configured to capture the molecules from the volume of the sample;

a desalting column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a second position different from the first position, the desalting column configured to reduce a salt concentration of the molecules;

a first reaction coil arranged to be selectively fluidly coupled to the first multi-port valve, the first reaction coil arranged to receive one or more of the change agents and configured to incubate the molecules and the one or more of the change agents to change the molecules; and

a second vial arranged downstream of and fluidly coupled to the second multi-port valve, the second vial arranged to receive a mixture comprising the molecules from the capture column, the desalting column, or the first reaction coil.

53. The closed system of claim 52, further comprising a needle configured to automatically retrieve the sample from the first vial and deposit the sample into the sample loop.

54. The closed system of claim 52 or 53, further comprising a third multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve, the third multi-port valve arranged to receive the one or more of the change agents via the first multi-port valve, and when the third multi-port valve is in a third position, the third-multi-port valve is arranged to direct the one or more change agents to the first reaction coil.

55. The closed system of any one of claims 52 to 54 further comprising a metering device fluidly coupled to the sample loop and the needle to control the volume of the sample obtained by the first multi-port valve.

56. The closed system of any one of claims 52 to 55, wherein the molecules comprise polypeptides, and wherein the capture column comprises a polypeptide-binding column.

57. The closed system of claim 56 wherein the polypeptide-binding column is selected from the group consisting of a protein A column, a protein G column, a protein A/G column, a protein L column, an amino acid column, an avidin column, a streptavidin column, a carbohydrate bonding column, a carbohydrate column, a glutathione column, a heparin column, a hydrophobic

interaction column, an immunoaffinity column, a nucleotide/coenzyme column, a specialty column, and an immobilized-metal affinity chromatography (IMAC) column.

58. The closed system of any one of claims 52 to 55, wherein the molecules comprise small molecules, and wherein the capture column comprises a reverse phase column.

59. The closed system of any one of claims 52 to 58, wherein the third multi-port valve comprises a 10-port valve.

60. The closed system of any one of claims 52 to 59, wherein the desalting column is arranged in parallel with the capture column.

61. The closed system of any one of claims 52 to 60, wherein the one or more change reagents are selected from the group consisting of a quenching reagent, a denaturing reagent, a reducing reagent, an alkylating reagent, a glycan labeling reagent, a solution containing glycosidases, an enzyme, and combinations thereof.

62. The closed system of claim 61, wherein the glycosidases are selected from the group consisting of glucanases, endoglycosidases, glycosamidases, and O-glycanases, and combinations thereof.

63. The closed system of claim 62, wherein the endoglycosidases are selected from the group consisting of endoglycosidase D, endoglycosidase F (endoglycosidase F1, endoglycosidase F2, and endoglycosidase F3 and combinations thereof), endoglycosidase H, endoglycosidase S, endoglycosidase M, and endoglycosidase B.

64. The closed system of claim 62, wherein the glycosamidases are selected from the group consisting of glycopeptidases, peptide N-glycosidases, PNGases, N-glycohydrolases, and N-glycanases.

65. The closed system of claim 50, wherein the PNGases is peptide:N-glycosidase F (PNGF).

66. The closed system of claim 62, wherein the O-glycanases comprise endo-GalNAc-ase D or endoGalNAc-ase A.

67. The closed system of any one of claims 61 to 66, wherein the glycan labeling reagent is a fluorophore or a chromophore.

68. The closed system of claim 67, wherein the fluorophore is selected from the group consisting of 2-aminobenzoic acid, 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt, 8-aminonaphthalene-1,3,6-trisulfonic acid trisodium salt, and anthranilamide, 4-methoxybenzamidine.

69. The closed system of claim 67, wherein the chromophore comprises 3-methyl-1-phenyl-2-pyrazoline-5-one or phenylhydrazine.

70. The closed system of any one of claims 52 to 69, further comprising a buffer source fluidly coupled to the first multi-port valve and arranged to supply buffer solution to the capture column when the second multi-port valve is in the first position, such that the elution buffer solution is adapted to elute substantially all of the molecules from the capture column.

71. The closed system of any one of claims 52 to 69, further comprising:

a glycosidase source arranged to supply glycosidases to the capture column via the second multi-port valve, such that the glycosidases are infused with the molecules captured by the capture column;

a carrier solution source arranged to supply a carrier solution to the capture column via the second multi-port valve, wherein the carrier solution is adapted to release the glycans from the capture column.

72. The closed system of any one of claims 52 to 71 further comprising an intermediate vial equipped with a mobile phase configured to reduce the salt concentration of the molecules in the second vial.

73. The closed system of any one of claims 52 to 72, further comprising a heating element positioned immediately adjacent the first reaction coil, the heating element configured to maintain the first reaction coil at a first pre-determined incubation temperature.

74. The closed system of claim 73, wherein the first pre-determined incubation temperature comprises approximately 30 degrees Celsius, approximately 40 degrees Celsius, or approximately 80 degrees Celsius.

75. The closed system of any one of claims 52 to 74, further comprising a second reaction coil arranged to be selectively fluidly coupled to the first multi-port valve, the second reaction coil arranged to receive one or more of the change agents and configured to incubate the polypeptides and the one or more of the change agents to change the molecules.

76. The closed system of claim 75, further comprising a heating element positioned immediately adjacent the second reaction coil, the heating element configured to maintain the second reaction coil at a second pre-determined incubation temperature.

77. The closed system of claim 76, wherein the second pre-determined incubation temperature comprises approximately 30 degrees Celsius, approximately 40 degrees Celsius, or approximately 80 degrees Celsius.

78. The closed system of any one of claims 52 to 77, further comprising a controller communicatively coupled to first and second multi-port valves.

79. The closed system of any one of claims 52 to 78, further comprising a first pump fluidly coupled to the first multi-port valve to facilitate movement of the sample throughout the closed system.

80. The closed system of claim 79, further comprising a second pump fluidly coupled to the second multi-port valve to facilitate cleaning of at least one of the capture column, the desalting column, and the first reaction coil.

81. The closed system of any one of claims 52 to 78, further comprising a first pump fluidly coupled to the second multi-port valve to facilitate cleaning of at least one of the capture column, the desalting column, and the first reaction coil.

82. A closed system, comprising:

a first vial adapted to contain a sample comprising polypeptides;

one or more change agents;

a sample loop adapted to receive the sample from the first vial;

a first multi-port valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a first port of the first multi-port valve;

a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve;

a capture column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a first position, the capture column configured to capture the polypeptides from the volume of the sample;

a desalting column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a second position different from the first position, the desalting column configured to reduce a salt concentration of the polypeptides;

a first reaction coil arranged to be selectively fluidly coupled to the first multi-port valve, the first reaction coil arranged to receive one or more of the change agents and configured to incubate the polypeptides and the one or more of the change agents to change the polypeptides; and

a second vial arranged downstream of and fluidly coupled to the second multi-port valve, the second vial arranged to receive a mixture comprising the polypeptides from the capture column, the desalting column, or the first reaction coil.

83. The closed system of claim 82, further comprising a needle configured to automatically retrieve the sample from the first vial and deposit the sample into the sample loop.

84. The closed system of claim 82 or 83, further comprising a third multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve, the third multi-port valve arranged to receive the one or more of the change agents via the first multi-port valve, and when the third multi-port valve is in a third position, the third-multi-port valve is arranged to direct the one or more change agents to the first reaction coil.

85. The closed system of any one of claims 82 to 84, further comprising a metering device fluidly coupled to the sample loop and the needle to control the volume of the sample obtained by the first multi-port valve.

86. The closed system of any one of claims 82 to 85, wherein the capture column comprises a polypeptide-binding column.

87. The closed system of claim 86, wherein the polypeptide-binding column is selected from the group consisting of a protein A column, a protein G column, a protein A/G column, a protein L column, an amino acid column, an avidin column, a streptavidin column, a carbohydrate bonding column, a carbohydrate column, a glutathione column, a heparin column, a hydrophobic interaction column, an immunoaffinity column, a nucleotide/coenzyme column, a specialty column, and an immobilized-metal affinity chromatography (IMAC) column.

88. The closed system of any one of claims 82 to 87, wherein the capture column is further configured to pre-concentrate the polypeptides.

89. The closed system of any one of claims 82 to 88, wherein the third multi-port valve comprises a 10-port valve.

90. The closed system of any one of claims 82 to 89, wherein the desalting column is arranged in parallel with the capture column.

91. The closed system of any one of claims 82 to 90, wherein the one or more change reagents are selected from the group consisting of a quenching reagent, a denaturing reagent, a reducing reagent, an alkylating reagent, a glycan labeling reagent, a solution containing glycosidases, an enzyme, and combinations thereof.

92. The closed system of claim 91, wherein the glycosidases are selected from the group consisting of endoglycosidases, glycosamidases, and O-glycanases, and combinations thereof.

93. The closed system of claim 92, wherein the endoglycosidases are selected from the group consisting of endoglycosidase D, endoglycosidase F (endoglycosidase F1, endoglycosidase F2, and endoglycosidase F3 and combinations thereof), endoglycosidase H, endoglycosidase S, endoglycosidase M, and endoglycosidase B.

94. The closed system of claim 92, wherein the glycosamidases are selected from the group consisting of glycopeptidases, peptide N-glycosidases, PNGases, N-glycohydrolases, and N-glycanases.

95. The closed system of claim 94, wherein the PNGases is peptide:N-glycosidase F (PNGF).

96. The closed system of claim 92, wherein the O-glycanases are endo-GalNAc-ase D or endoGalNAc-ase A.

97. The closed system of any one of claims 91 to 96, wherein the glycan labeling reagent is a fluorophore or a chromophore.

98. The closed system of claim 97, wherein the fluorophore is selected from the group consisting of 2-aminobenzoic acid, 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt, 8-aminonaphthalene-1,3,6-trisulfonic acid trisodium salt, and anthranilamide, 4-methoxybenzamidine.

99. The closed system of claim 97, wherein the chromophore comprises 3-methyl-1-phenyl-2-pyrazoline-5-one or phenylhydrazine.

100. The closed system of any one of claims 82 to 99, further comprising a buffer source fluidly coupled to the first multi-port valve and arranged to supply buffer solution to the capture column when the second multi-port valve is in the first position, such that the elution buffer solution is adapted to elute substantially all of the polypeptides from the capture column.

101. The closed system of any one of claims 82 to 99, further comprising:

a glycosidase source arranged to supply glycosidases to the capture column via the second multi-port valve, such that the glycosidases are infused with the captured by the capture column;

a carrier solution source arranged to supply a carrier solution to the capture column via the second multi-port valve, wherein the carrier solution is adapted to release the glycans from the capture column.

102. The closed system of any one of claims 82 to 101 further comprising an intermediate vial equipped with a mobile phase configured to reduce the salt concentration of the polypeptides in the second vial.

103. The closed system of any one of claims 82 to 102, further comprising a heating element positioned immediately adjacent the first reaction coil, the heating element configured to maintain the first reaction coil at a first pre-determined incubation temperature.

104. The closed system of claim 103, wherein the first pre-determined incubation temperature comprises approximately 30 degrees Celsius, approximately 40 degrees Celsius, or approximately 80 degrees Celsius.

105. The closed system of any one of claims 82 to 104, further comprising a second reaction coil arranged to be selectively fluidly coupled to the first multi-port valve, the second reaction coil arranged to receive one or more of the change agents and configured to incubate the polypeptides and the one or more of the change agents to change the polypeptides.

106. The closed system of claim 105, further comprising a heating element positioned immediately adjacent the second reaction coil, the heating element configured to maintain the second reaction coil at a second pre-determined incubation temperature.

107. The closed system of claim 106, wherein the second pre-determined incubation temperature comprises approximately 30 degrees Celsius, approximately 40 degrees Celsius, or approximately 80 degrees Celsius.

108. The closed system of any one of claims 82 to 107, further comprising a controller communicatively coupled to the first and second multi-port valves.

109. The closed system of any one of claims 82 to 108, further comprising a first pump fluidly coupled to the first multi-port valve to facilitate movement of the sample throughout the closed system.

110. The closed system of claim 109, further comprising a second pump fluidly coupled to the second multi-port valve to facilitate cleaning of at least one of the capture column, the desalting column, and the first reaction coil.

111. The closed system of any one of claims 82 to 108, further comprising a first pump fluidly coupled to the second multi-port valve to facilitate cleaning of at least one of the capture column, the desalting column, and the first reaction coil.

112. A closed system comprising:

a first vial adapted to contain a sample comprising molecules;

a sample loop adapted to receive the sample from the first vial;

a first multi-port valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a first port of the first multi-port valve;

a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve;

a capture column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a first position, the capture column configured to bind the molecules from the sample;

a second vial arranged downstream of the capture column; and

a buffer source fluidly coupled to the first multi-port valve and arranged to supply elution buffer solution to the second vial via the second multi-port valve and the capture column when the second multi-port valve is in the first position, such that the elution buffer solution is adapted to elute substantially all of the molecules from the capture column,

wherein the second vial comprises a flow-through vial configured to filter the elution buffer solution from the elution/molecule mixture out of the second vial, thereby leaving only the eluted molecules in the second vial.

113. The closed system of claim 112, further comprising a needle configured to automatically retrieve the sample from the first vial and deposit the sample into the sample loop.

114. The closed system of claim 112 or 113, further comprising a metering device fluidly coupled to the sample loop and the needle to control the volume of the sample obtained by the first multi-port valve.

115. The closed system of any one of claims 112 to 114, wherein the molecules comprise metabolites, and the capture column comprises a tri-mode chromatography column.

116. The closed system of claim 115, wherein the elution buffer solution comprises a volatile buffer solution.

117. The closed system of any one of claims 112 to 114, wherein the molecules comprise polypeptides, and the capture column comprises a polypeptide binding column.

118. The closed system of any one of claims 112 to 114, wherein the polypeptide binding column is further configured to pre-concentrate the polypeptides.

119. The closed system of any one of claims 112 to 118, further comprising a desalting column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a second position different from the first position, wherein when the second multi-port valve is in the second position, the desalting column is arranged to receive the eluted polypeptides in the second vial and configured to reduce a salt concentration of the eluted polypeptides.

120. The closed system of any one of claims 112 to 119, further comprising an intermediate vial equipped with a mobile phase configured to reduce the salt concentration of the eluted polypeptides in the second vial.

120. The closed system of any one of claims 112 to 120, further comprising:

a quenching reagent; and

a first reaction coil configured to receive the eluted polypeptides from the second valve and the quenching reagent via the first multi-port valve, the reaction coil configured to incubate the eluted polypeptides with the quenching reagent to quench the polypeptides.

121. The closed system of any one of claims 112 to 120, further comprising:

a denaturing reagent in the second vial;

a reducing reagent in the second vial;

a first reaction coil configured to receive the eluted polypeptides, the denaturing reagent, and the reducing reagent from the second vial via the first multi-port valve, the first reaction coil configured to incubate the eluted polypeptides with the denaturing reagent and the reducing reagent to produce denatured and reduced polypeptides;

a third vial arranged to receive the denatured and reduced polypeptides and comprising an alkylating reagent,

wherein the first reaction coil is further arranged to receive the alkylating reagent and the denatured and reduced polypeptides from the third vial, the first reaction coil further configured to incubate the alkylating reagent with the denatured and reduced polypeptides to alkylate the denatured and reduced polypeptides, and

wherein the second vial is further arranged to receive the denatured, reduced, and alkylated polypeptides.

122. The closed system of claim 121, further comprising a desalting column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a second position different from the first position, wherein when the second multi-port valve is in the second position, the desalting column is arranged to receive the denatured, reduced, and alkylated polypeptides from the second vial, the desalting column configured to desalt the denatured, reduced, and alkylated polypeptides, and wherein the second vial is further arranged to receive the desalted polypeptides from the desalting column.

123. The closed system of claim 122, further comprising an enzyme in the second vial and a second reaction coil arranged to receive the desalted polypeptides and the enzyme from the second valve via the first multi-port valve, the second reaction

coil configured to incubate the desalted polypeptides and the enzyme to digest the polypeptides, wherein the third vial is further arranged to receive the digested polypeptides from the second reaction coil.

124. A closed system comprising:

a first vial adapted to contain a sample comprising polypeptides;

a sample loop adapted to receive the sample from the first vial;

a first multi-port valve fluidly coupled to the sample loop and arranged to obtain a volume of the sample via a first port of the first multi-port valve;

a second multi-port valve fluidly coupled to and arranged downstream of the first multi-port valve;

a polypeptide-binding column arranged to be fluidly coupled to the second multi-port valve when the second multi-port valve is in a first position, the polypeptide binding column configured to bind the polypeptides from the sample;

a second vial arranged downstream of the polypeptide-binding column, the second vial being a flow-through vial and comprising a glycan-labeling reagent;

a glycosidase source arranged to supply glycosidases to the polypeptide-binding column via the second multi-port valve, such that the glycosidases are infused with the polypeptides bound to the polypeptide-binding column;

a carrier solution source arranged to supply a carrier solution to the polypeptide-binding column via the second multi-port valve, wherein the carrier solution is adapted to release and carry the glycans from the polypeptide-binding column and to the second vial;

a reaction coil arranged to receive a mixture of the released glycans and the glycan-labeling reagent from the second vial via the first multi-port valve, the reaction coil configured to incubate the mixture of the released glycans and the glycan-labeling reagent to label the glycans;

a third vial arranged downstream of the reaction coil to receive the labeled glycans and the glycan-labeling reagent.

125. The closed system of claim 124, further comprising a needle configured to automatically retrieve the sample from the first vial and deposit the sample into the sample loop.

126. The closed system of claim 124 or 125, wherein the glycosidases are selected from the group consisting of endoglycosidases, glycosamidases, and O-glycanases, and combinations thereof.

127. The closed system of claim 126, wherein the endoglycosidases are selected from the group consisting of endoglycosidase D, endoglycosidase F (endoglycosidase F1, endoglycosidase F2, and endoglycosidase F3 and combinations thereof), endoglycosidase H, endoglycosidase S, endoglycosidase M, and endoglycosidase B.

128. The closed system of claim 126, wherein the glycosamidases are selected from the group consisting of glycopeptidases, peptide N-glycosidases, PNGases, N-glycohydrolases, and N-glycanases.

129. The closed system of claim 128, wherein the PNGases is peptide:N-glycosidase F (PNGF).

130. The closed system of claim 126, wherein the O-glycanases comprise endo-GalNAc-ase D or endoGalNAc-ase A.

131. The closed system of any one of claims 124 to 130, wherein the glycan labeling reagent is a fluorophore or a chromophore.

132. The closed system of claim 131, wherein the fluorophore is selected from the group consisting of 2-aminobenzoic acid, 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt, 8-aminonaphthalene-1,3,6-trisulfonic acid trisodium salt, and anthranilamide, 4-methoxybenzamidine.

133. The closed system of claim 131, wherein the chromophore comprises 3-methyl-1-phenyl-2-pyrazoline-5-one or phenylhydrazine.

134. The closed system of any one of claims 124 to 133, further comprising a heating element positioned immediately adjacent the reaction coil, the heating element configured to maintain the reaction coil at a temperature of approximately 80 degrees Celsius.

135. The closed system of any one of claims 52 to 134, further comprising an analytical device.

136. The closed system of claim 135, wherein the analytical device comprises an IEX,CEX, or AEX chromatographic column.

137. The closed system of any one of claims 52 to 136, wherein the first multi-port valve comprises a 6 port valve.

138. The closed system of any one of claims 52 to 137, wherein the second multi-port valve comprises a 10 port valve.

139. The closed system of any one of claims 124 to 138, further comprising a controller communicatively coupled to the needle and the first and second multi-port valves.

140. The closed system of any one of claims 124 to 139, wherein the polypeptide-binding column is selected from the group consisting of a protein A column, a protein G column, a protein A/G column, a protein L column, an amino acid column, an avidin column, a streptavidin column, a carbohydrate bonding column, a carbohydrate column, a glutathione column, a heparin column, a hydrophobic interaction column, an immunoaffinity column, a nucleotide/coenzyme column, a specialty column, and an immobilized-metal affinity chromatography (IMAC) column.

141. The closed system of any one of claims 124 to 140, further comprising a first pump fluidly coupled to the first multi-port valve to facilitate movement of the sample throughout the closed system.

142. The closed system of claim 141, further comprising a second pump fluidly coupled to the second multi-port valve to facilitate cleaning of at least one of the capture column, the desalting column, and the first reaction coil.

143. The method of claims 13 and 19 to 39 or the closed system of claims 56, 57, 59 to 111, and 117 to 142, wherein the polypeptide of the product is a therapeutic polypeptide.

144. The method or closed system of claim 143, wherein the therapeutic polypeptide is selected from the group consisting of an antibody or antigen-binding fragment thereof, a derivative of an antibody or antibody fragment, and a fusion polypeptide.

145. The method or closed system of claim 144, wherein the antibody is selected from the group consisting of infliximab, bevacizumab, ranibizumab, cetuximab, ranibizumab, palivizumab, abagovomab, abciximab, actoxumab, adalimumab, afelimomab, afutuzumab, alacizumab, alacizumab pegol, ald518, alemtuzumab, alirocumab, alemtuzumab, altumomab, amatuximab, anatumomab mafenatox, anrukinzumab, apolizumab, arcitumomab, aselizumab, altinumab, atlizumab, atorolimiumab, tocilizumab, bapineuzumab, basiliximab, bavituximab, bectumomab, belimumab, benralizumab, bertilimumab, besilesomab, bevacizumab, bezlotoxumab, biciromab, bivatuzumab, bivatuzumab mertansine, blinatumomab, blosozumab, brentuximab vedotin, briakinumab, brodalumab, canakinumab, cantuzumab mertansine, cantuzumab mertansine, caplacizumab, capromab pendetide, carlumab, catumaxomab, cc49, cedelizumab, certolizumab pegol, cetuximab, citatuzumab bogatox, cixutumumab, clazakizumab, clenoliximab, clivatuzumab tetraxetan, conatumumab, crenezumab, cr6261, dacetuzumab, daclizumab, dalotuzumab, daratumumab, demcizumab, denosumab, detumomab, dorlimomab aritox, drozitumab, duligotumab, dupilumab, ecromeximab, eculizumab, edobacomab, edrecolomab, efalizumab, efungumab, elotuzumab, elsilimomab, enavatuzumab, enlimomab pegol, enokizumab, enokizumab, enoticumab, enoticumab, ensituximab, epitumomab cituxetan, epratuzumab, erlizumab, ertumaxomab, etaracizumab, etrolizumab, exbivirumab, exbivirumab, fanolesomab, faralimomab, farletuzumab, fasinumab, fbta05, felvizumab, fezakinumab, ficlatuzumab, figitumumab, flanvotumab, fontolizumab, foralumab, foravirumab, fresolimumab, fulranumab, futuximab, galiximab, ganitumab, gantenerumab, gavilimomab, gemtuzumab ozogamicin, gevokizumab, girentuximab, glembatumumab vedotin, golimumab, gomiliximab, gs6624, ibalizumab, ibritumomab tiuxetan, icrucumab, igovomab, imciromab, imgatuzumab, inclacumab, indatuximab ravtansine, infliximab, intetumumab, inolimomab, inotuzumab ozogamicin, ipilimumab, iratumumab, itolizumab, ixekizumab, keliximab, labetuzumab, lebrikizumab, lemalesomab, lerdelimumab, lexatumumab, libivirumab, ligelizumab, lintuzumab, lirilumab, lorvotuzumab mertansine, lucatumumab, lumiliximab, mapatumumab, maslimomab, mavrilimumab, matuzumab, mepolizumab, metelimumab, milatuzumab, minretumomab, mitumomab, mogamulizumab, morolimumab, motavizumab, moxetumomab pasudotox, muromonab-cd3, nacolomab tafenatox, namilumab, naptumomab estafenatox, narnatumab, natalizumab, nebacumab, necitumumab, nerelimomab, nesvacumab, nimotuzumab, nivolumab, nofetumomab merpentan, ocaratuzumab, ocrelizumab, odulimomab, ofatumumab, olaratumab, olokizumab, omalizumab, onartuzumab, oportuzumab monatox, oregovomab, orticumab, otelixizumab, oxelumab, ozanezumab, ozoralizumab, pagibaximab, palivizumab, panitumumab, panobacumab, parsatuzumab, pascolizumab, pateclizumab, patritumab, pemtumomab, perakizumab, pertuzumab, pexelizumab, pidilizumab, pintumomab, placulumab, ponezumab, priliximab, pritumumab, PRO 140, quilizumab, racotumomab, radretumab, rafivirumab, ramucirumab, ranibizumab, raxibacumab, regavirumab, reslizumab, rilotumumab, rituximab, robatumumab, roledumab, romosozumab, rontalizumab, rovelizumab, ruplizumab, samalizumab, sarilumab, satumomab pendetide, secukinumab, sevirumab, sibrotuzumab, sifalimumab,

siltuximab, simtuzumab, siplizumab, sirukumab, solanezumab, solitomab, sonepcizumab, sontuzumab, stamulumab, sulesomab, suvizumab, tabalumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tanezumab, taplitumomab paptox, tefibazumab, telimomab aritox, tenatumomab, tefibazumab, telimomab aritox, tenatumomab, teneliximab, teplizumab, teprotumumab, tezepelumab, TGN1412, tremelimumab, ticilimumab, tildrakizumab, tigatuzumab, TNX-650, tocilizumab, toralizumab, tositumomab, tralokinumab, trastuzumab, TRBS07, tregalizumab, tremelimumab, tucotuzumab celmoleukin, tuvirumab, ublituximab, urelumab, urtoxazumab, ustekinumab, vapaliximab, vatelizumab, vedolizumab, veltuzumab, vepalimomab, vesencumab, visilizumab, volociximab, vorsetuzumab mafodotin, votumumab, zalutumumab, zanolimumab, zatuximab, ziralimumab, zolimomab aritox, and those antibodies shown in Table 1.

146. The method or closed system of claim 143, wherein the therapeutic polypeptide is a polypeptide selected from the group consisting of a glycoprotein, CD polypeptide, a HER receptor polypeptide, a cell adhesion polypeptide, a growth factor polypeptide, an insulin polypeptide, an insulin-related polypeptide, a coagulation polypeptide, a coagulation-related polypeptide, albumin, IgE, a blood group antigen, a colony stimulating factor, a receptor, a neurotrophic factor, an interferon, an interleukin, a viral antigen, a lipoprotein, calcitonin, glucagon, atrial natriuretic factor, lung surfactant, tumor necrosis factor-alpha and -beta, enkephalinase, mouse gonadotropin-associated peptide, DNAse, inhibin, activing, an integrin, protein A, protein D, a rheumatoid factor, an immunotoxin, a bone morphogenetic protein, a superoxide dismutase, a surface membrane polypeptide, a decay accelerating factor, an AIDS envelope, a transport polypeptide, a homing receptor, an addressin, a regulatory polypeptide, an immunoadhesin, a myostatin, a TALL polypeptide, an amyloid polypeptide, a thymic stromal lymphopoietin, a RANK ligand, a c-kit polypeptide, a TNF receptor, and an angiopoietin, and biologically active fragments, analogs or variants thereof.

147. The closed system of any one of claims 52 to 146, wherein the flow-through vial comprises a base, a gutter coupled to and extending outwardly from the base, an inlet port formed in the base, and an outlet port formed in the gutter.

148. The closed system of claim 147, wherein the flow-through vial further comprises a lip arranged between the base and the gutter, the lip configured to reduce surface tension of fluid flowing through the flow-through vial.

149. The closed system of claim 147 or 148, wherein the inlet port extends along an inlet axis and the outlet port extends along an outlet axis, the outlet axis being inclined relative to the inlet axis.

150. The closed system of claim 149, wherein the base extends along a longitudinal axis that is inclined relative to each of the inlet axis and the outlet axis.

151. The closed system of any one of claims 147 to 150, wherein the gutter comprises a downspout, and wherein the outlet port is formed in the downspout.

152. The closed system of any one of claims 52 to 151, further comprising a vial holder configured to receive and retain the flow-through vial.

153. The closed system of claim 152, wherein the vial holder comprises a plurality of receptacles configured to receive and retain a plurality of flow-through vials.

154. A flow-through vial for use in preparing a sample of a product containing molecules for analysis and performing an assay of that sample, the flow-through vial comprising:

a base;

a gutter coupled to and extending outwardly from the base;

an inlet port formed in the base; and

an outlet port formed in the gutter.

155. The flow-through vial of claim 154, wherein the flow-through vial further comprises a lip arranged between the base and the gutter, the lip configured to reduce surface tension of fluid flowing through the flow-through vial.

156. The flow-through vial of claim 154 or claim 155, wherein the inlet port extends along an inlet axis and the outlet port extends along an outlet axis, the outlet axis being inclined relative to the inlet axis.

157. The flow-through vial of claim 156, wherein the base extends along a longitudinal axis that is inclined relative to each of the inlet axis and the outlet axis.

158. The flow-through vial of any one of claims 154 to 157, wherein the gutter comprises a downspout, and wherein the outlet port is formed in the downspout.

159. The closed system of any one of clams 52 to 111, or 143 to 153, wherein the second vial comprises a flow-through vial configured to separate the molecules from a remainder of the mixture, such that the molecules can be analyzed.

160. The closed system of any one of claims 124 to 153, wherein the third vial is also a flow-through vial configured to substantially filter the glycan-labeling reagent out of the third vial, thereby leaving substantially only the labeled glycans in the third vial.

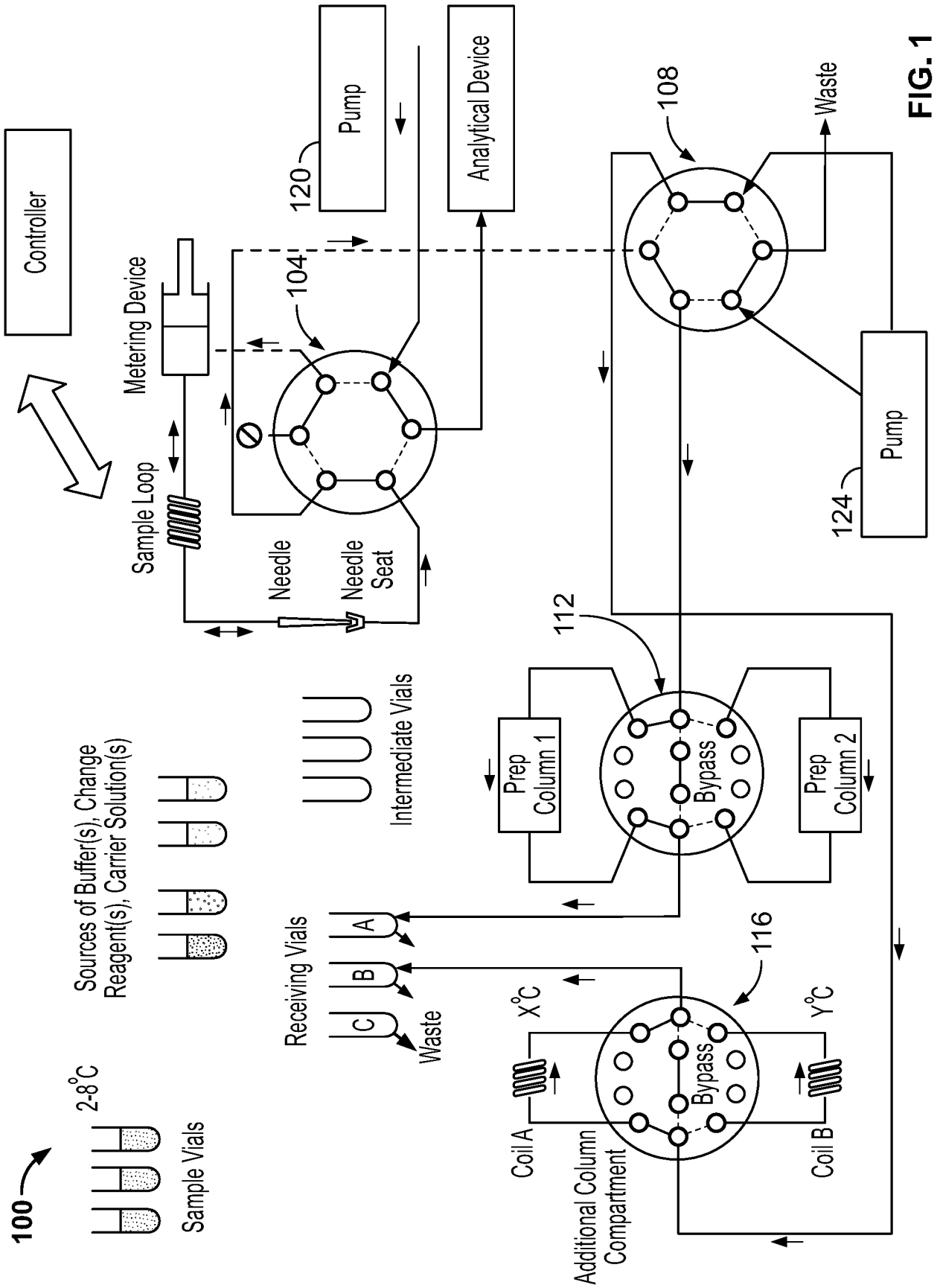

100
2-8°C
Sample Vials
Sources of Buffer(s), Change Reagent(s), Carrier Solution(s)
Receiving Vials
A
B
C
Waste
Intermediate Vials
Controller
Metering Device
Sample Loop
Needle
Needle Seat
104
120
Pump
Analytical Device
108
Waste
124
Pump
112
Prep Column 1
Bypass
Prep Column 2
116
X°C
Y°C
Coil A
Coil B
Additional Column Compartment
Bypass

FIG. 1

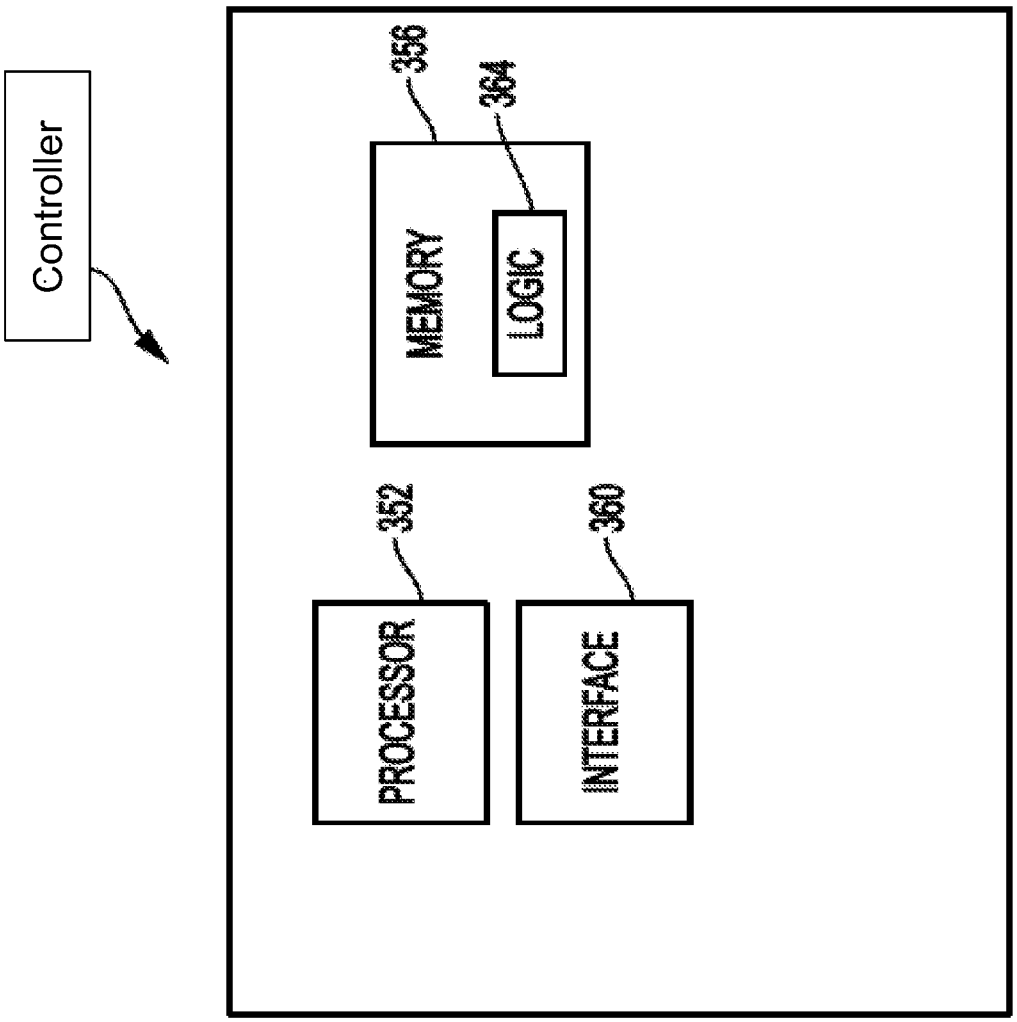

Controller
356
364
MEMORY
LOGIC
352
360
PROCESSOR
INTERFACE

FIG. 2

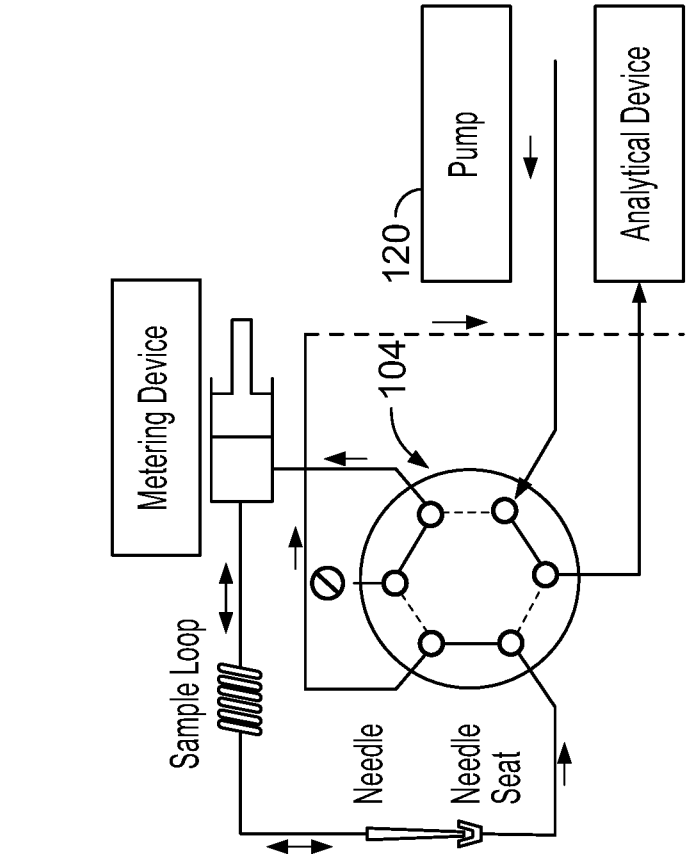
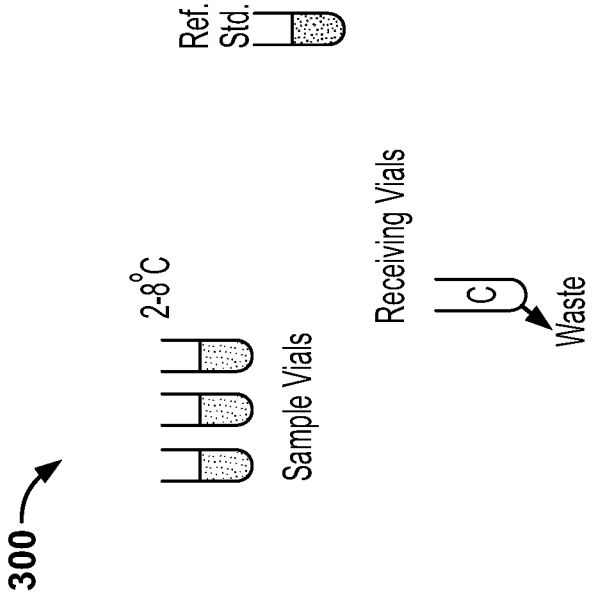

Pump
120
Analytical Device
Metering Device
104
Sample Loop
Needle
Needle Seat

FIG. 3


Ref. Std.
2-8°C
Receiving Vials
Waste
Sample Vials
300

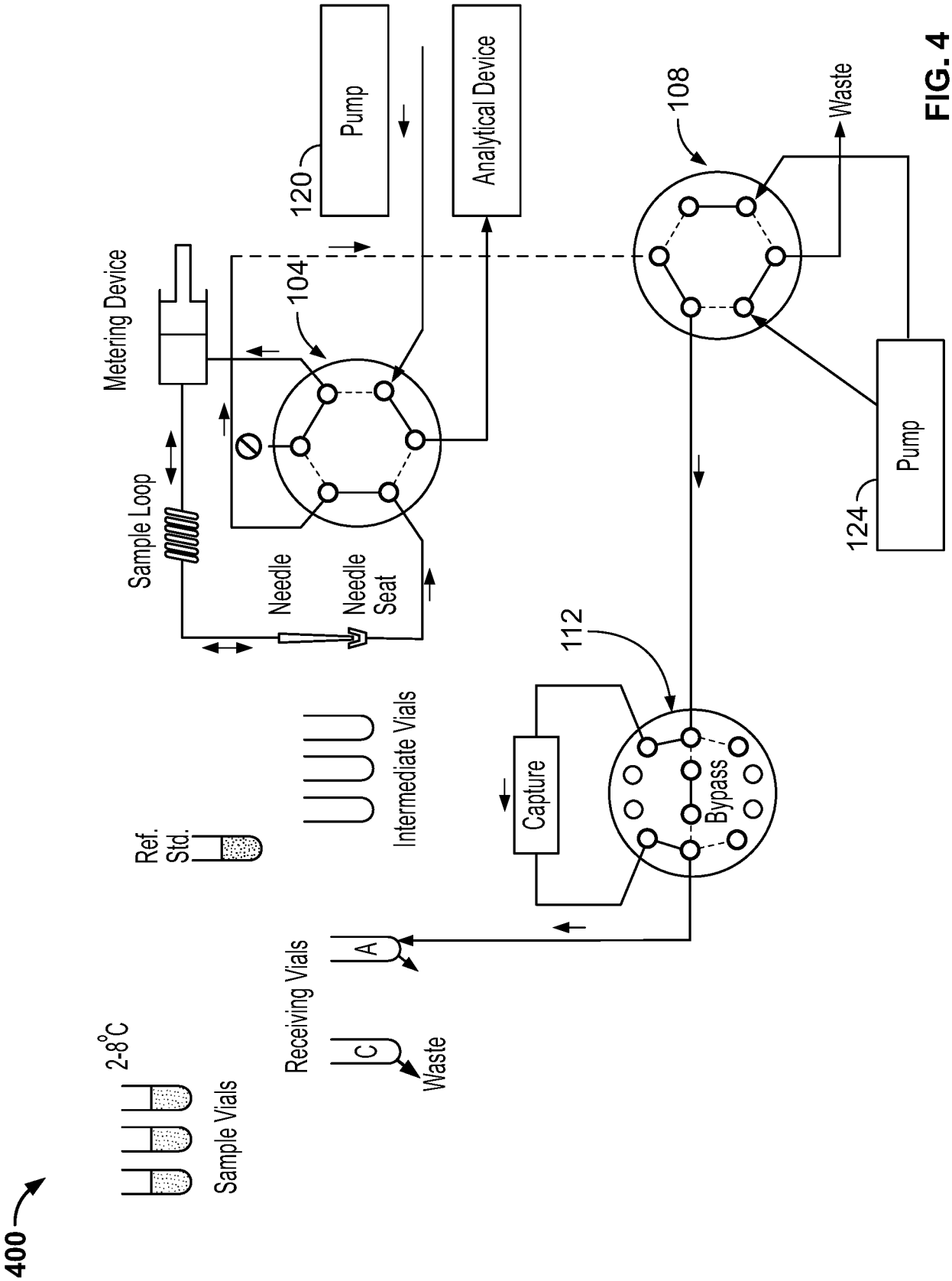

400
2-8°C
Sample Vials
Receiving Vials
A
C
Waste
Ref. Std.
Intermediate Vials
Capture
112
Bypass
Sample Loop
Needle
Needle Seat
Metering Device
104
120
Pump
Analytical Device
108
Waste
124
Pump

FIG. 4

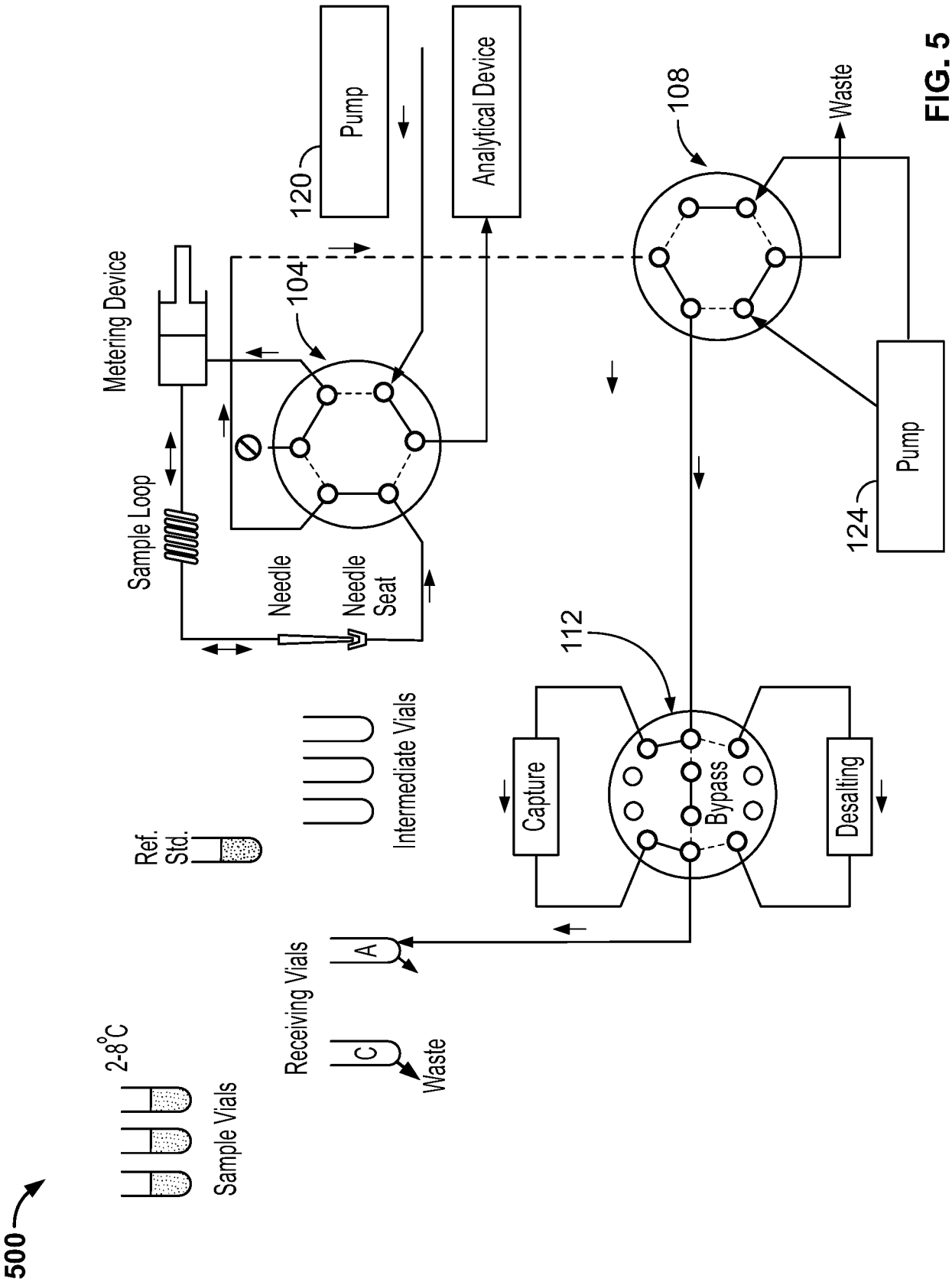

500
2-8°C
Sample Vials
Receiving Vials
A
C
Waste
Ref. Std.
Intermediate Vials
Metering Device
Sample Loop
Needle
Needle Seat
104
120
Pump
Analytical Device
108
Waste
112
Capture
Bypass
Desalting
124
Pump

FIG. 5

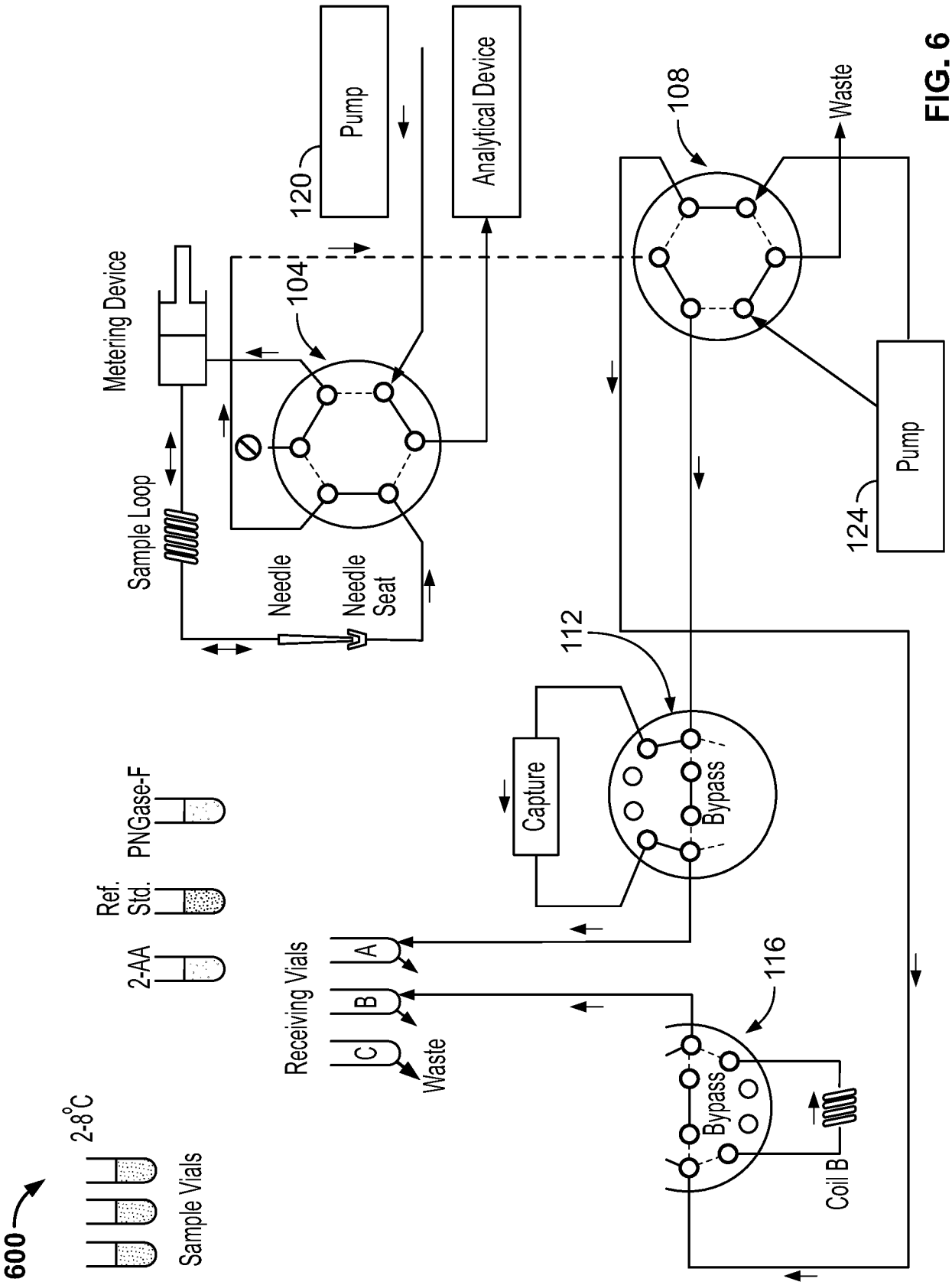

600
2-8°C
Sample Vials
2-AA
Ref. Std.
PNGase-F
Receiving Vials
A
B
C
Waste
Metering Device
Sample Loop
Needle
Needle Seat
104
120
Pump
Analytical Device
108
Waste
124
Pump
112
Capture
Bypass
116
Bypass
Coil B

FIG. 6

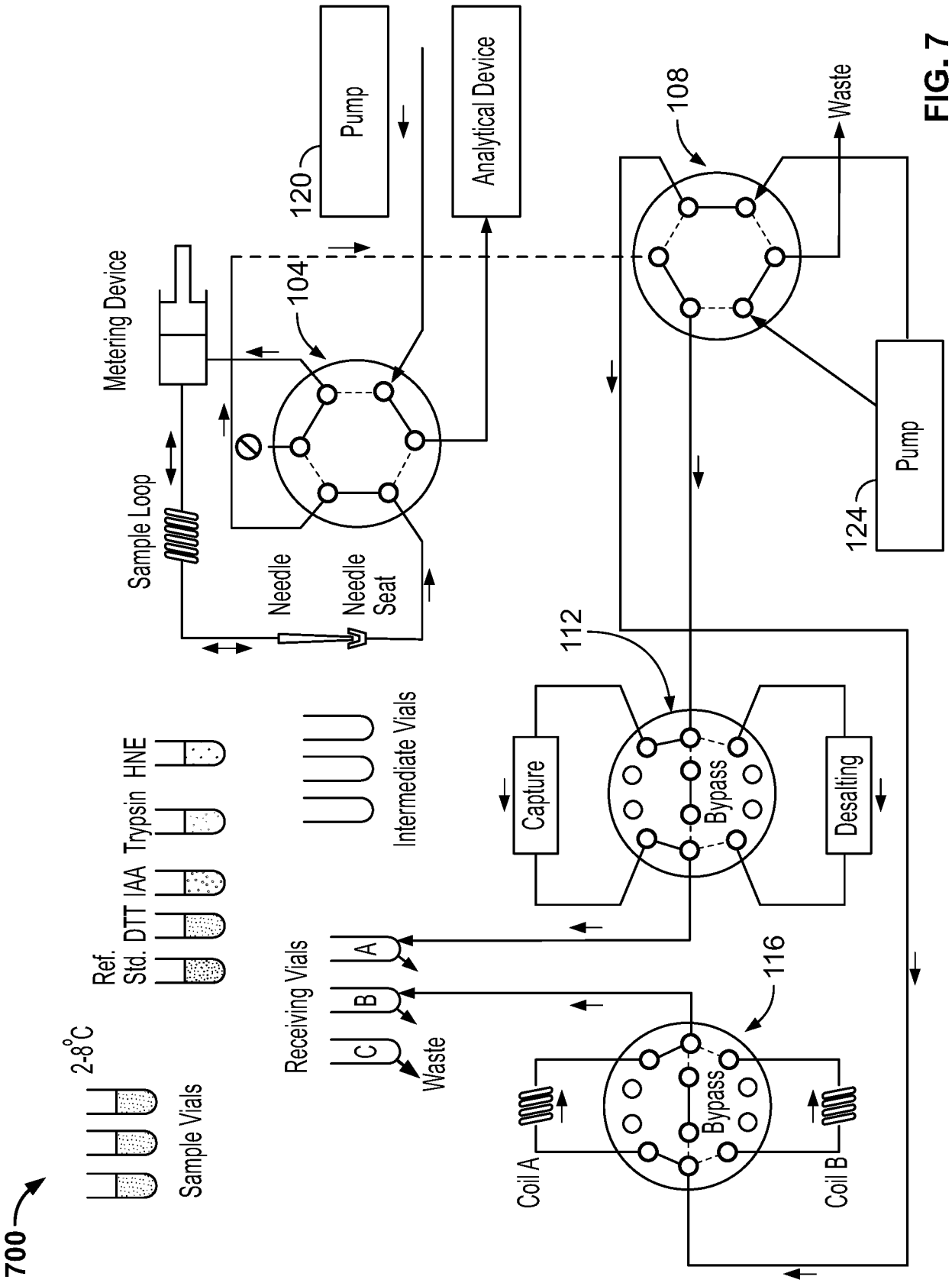

700
2-8°C
Sample Vials
Ref. Std.
DTT
IAA
Trypsin
HNE
Intermediate Vials
Receiving Vials
A
B
C
Waste
Metering Device
Sample Loop
Needle
Needle Seat
104
120
Pump
Analytical Device
108
Waste
124
Pump
112
Capture
Bypass
Desalting
116
Bypass
Coil A
Coil B

FIG. 7

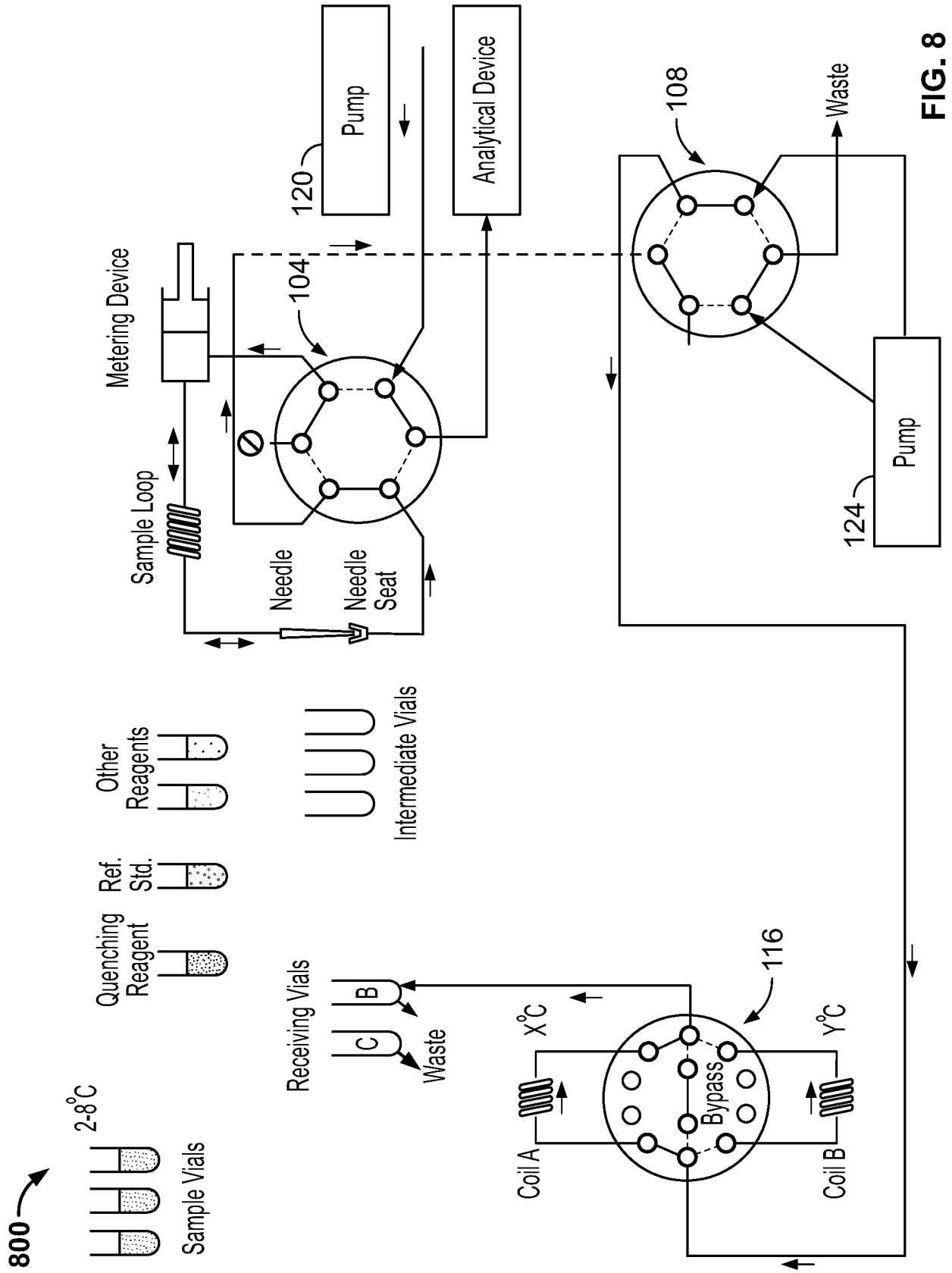

800
2-8°C
Sample Vials
Quenching Reagent
Ref. Std.
Other Reagents
Intermediate Vials
Receiving Vials
B
C
Waste
X°C
Y°C
Coil A
Coil B
Bypass
116
Sample Loop
Metering Device
Needle
Needle Seat
104
120
Pump
Analytical Device
108
Waste
124
Pump

FIG. 8

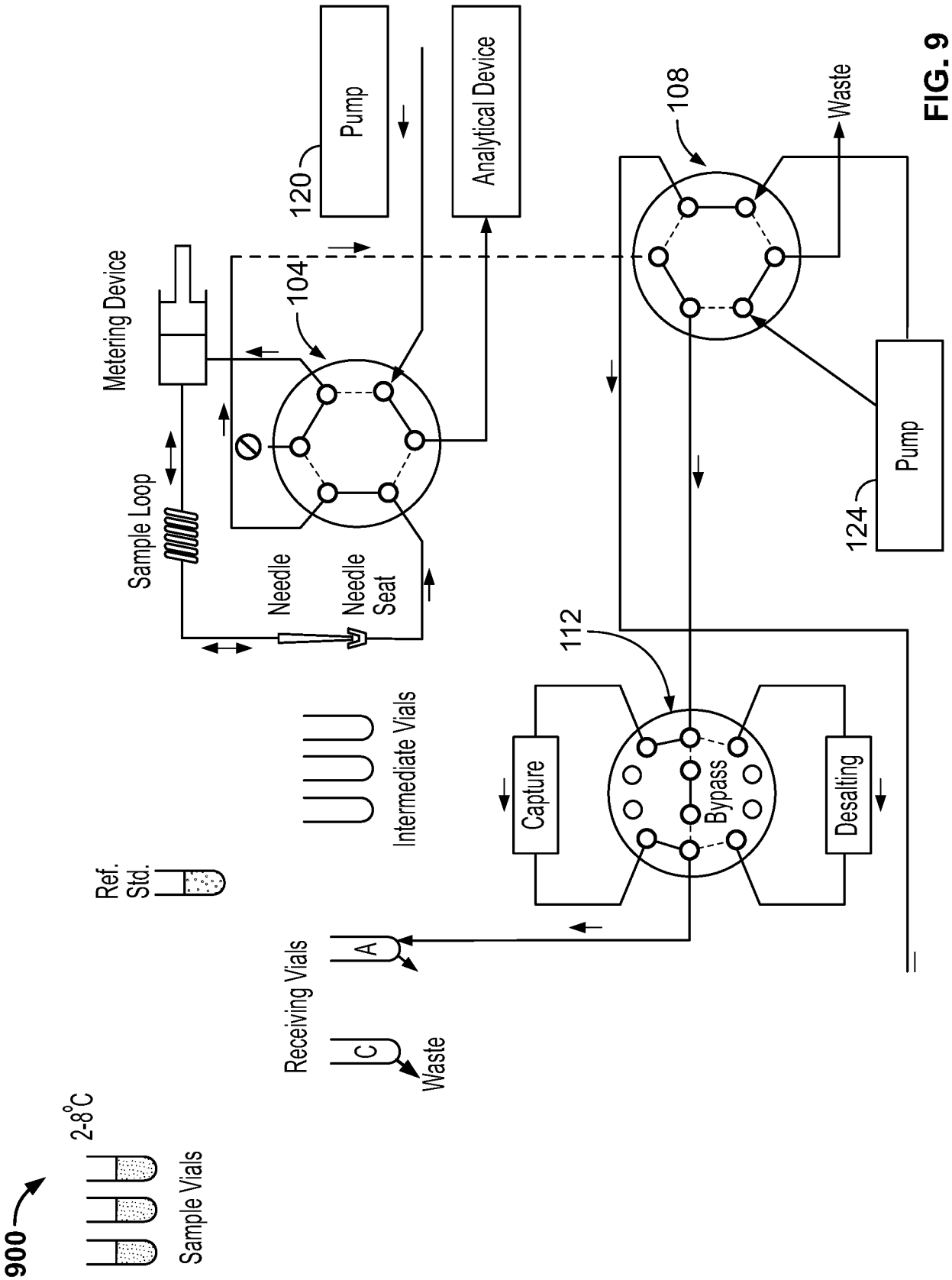

900
2-8°C
Sample Vials
Ref. Std.
Receiving Vials
A
C
Waste
Intermediate Vials
Metering Device
Sample Loop
Needle
Needle Seat
104
120
Pump
Analytical Device
108
Waste
112
Capture
Bypass
Desalting
124
Pump

FIG. 9

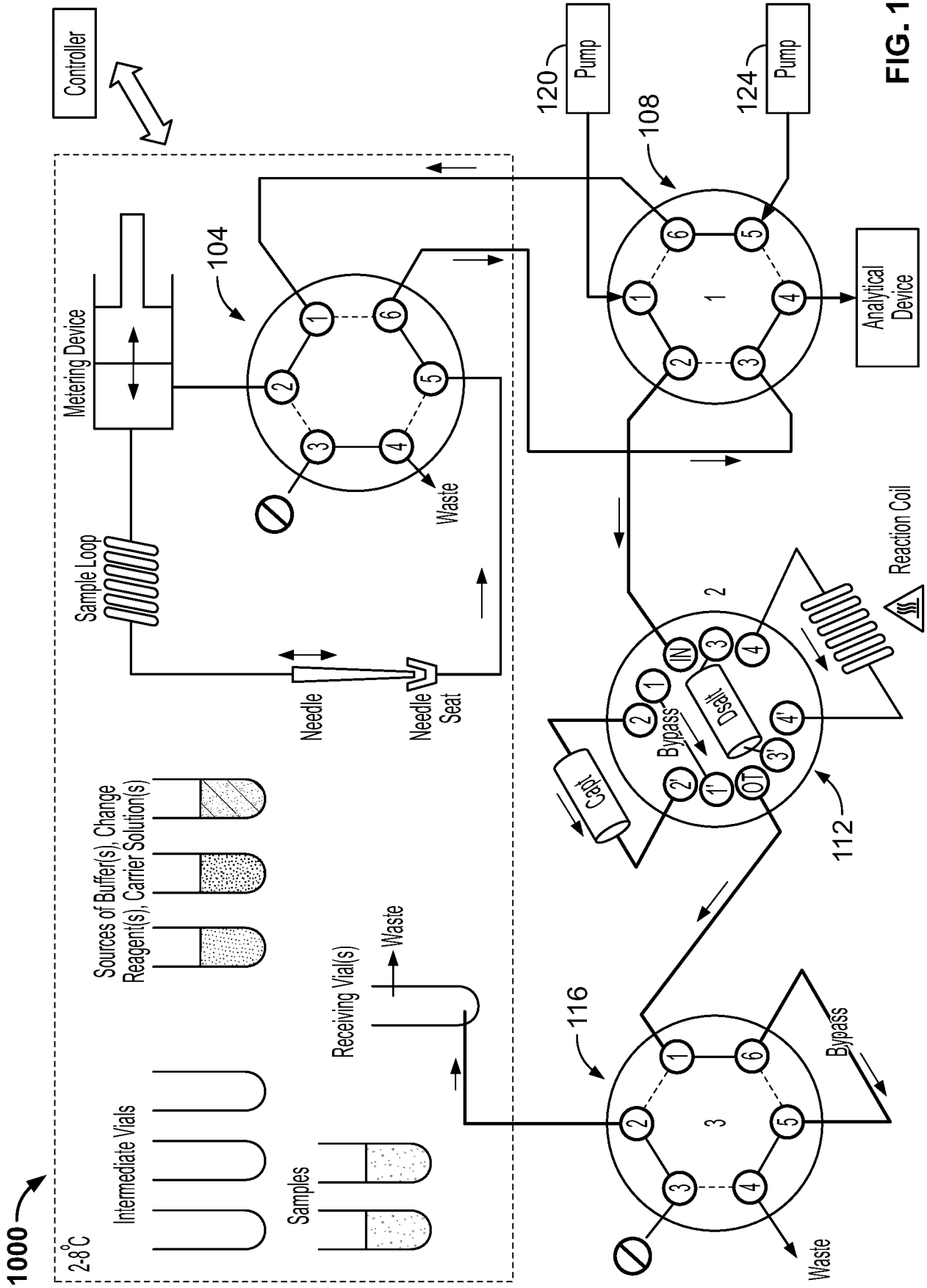

1000
2-8°C
Controller
Metering Device
104
Sample Loop
Needle
Needle Seat
Waste
Sources of Buffer(s), Change Reagent(s), Carrier Solution(s)
Intermediate Vials
Samples
Receiving Vial(s)
Waste
116
120
Pump
124
Pump
108
Analytical Device
Reaction Coil
Bypass
Dsalt
Calt
IN
OT
112
Bypass
Waste

FIG. 10

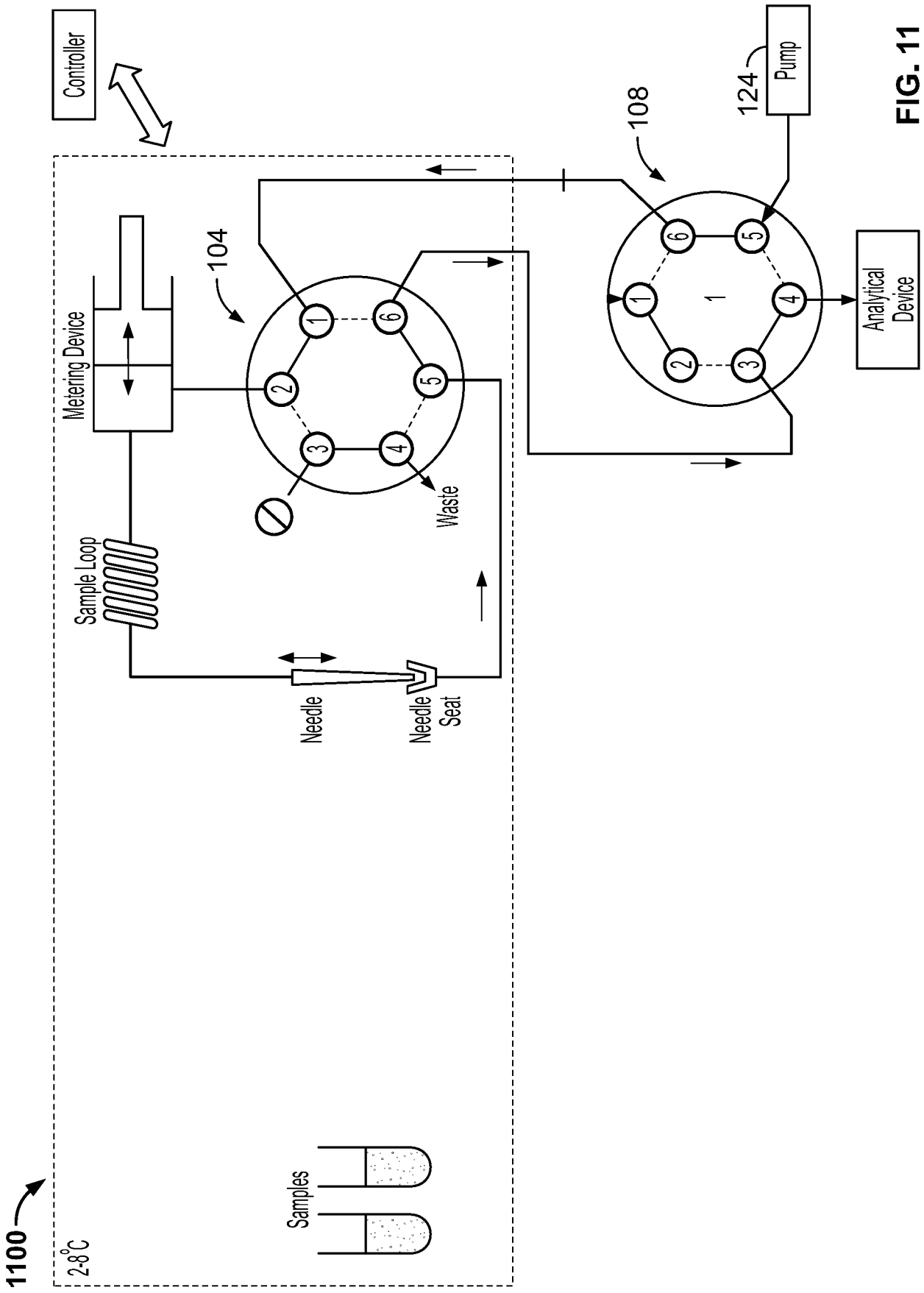

Controller
Pump
124
108
104
Metering Device
Analytical Device
Sample Loop
Waste
Needle
Needle Seat
Samples
1100
2-8°C

FIG. 11

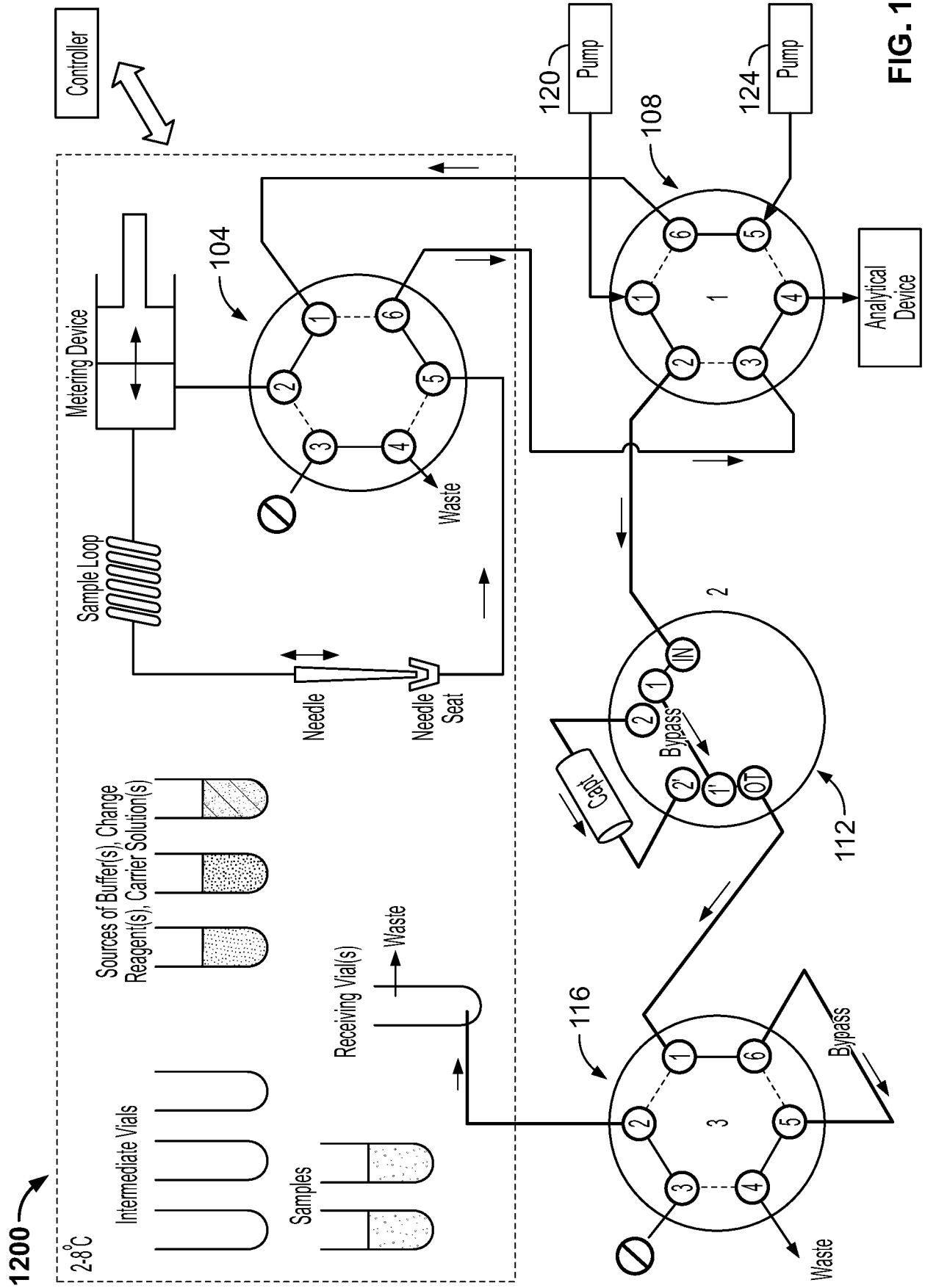

Controller
120
Pump
124
Pump
108
104
Metering Device
Sample Loop
Needle
Needle Seat
Waste
Analytical Device
Bypass
Cal
112
Sources of Buffer(s), Change Reagent(s), Carrier Solution(s)
Receiving Vial(s)
Waste
116
Bypass
Intermediate Vials
Samples
2-8°C
1200
Waste

FIG. 12

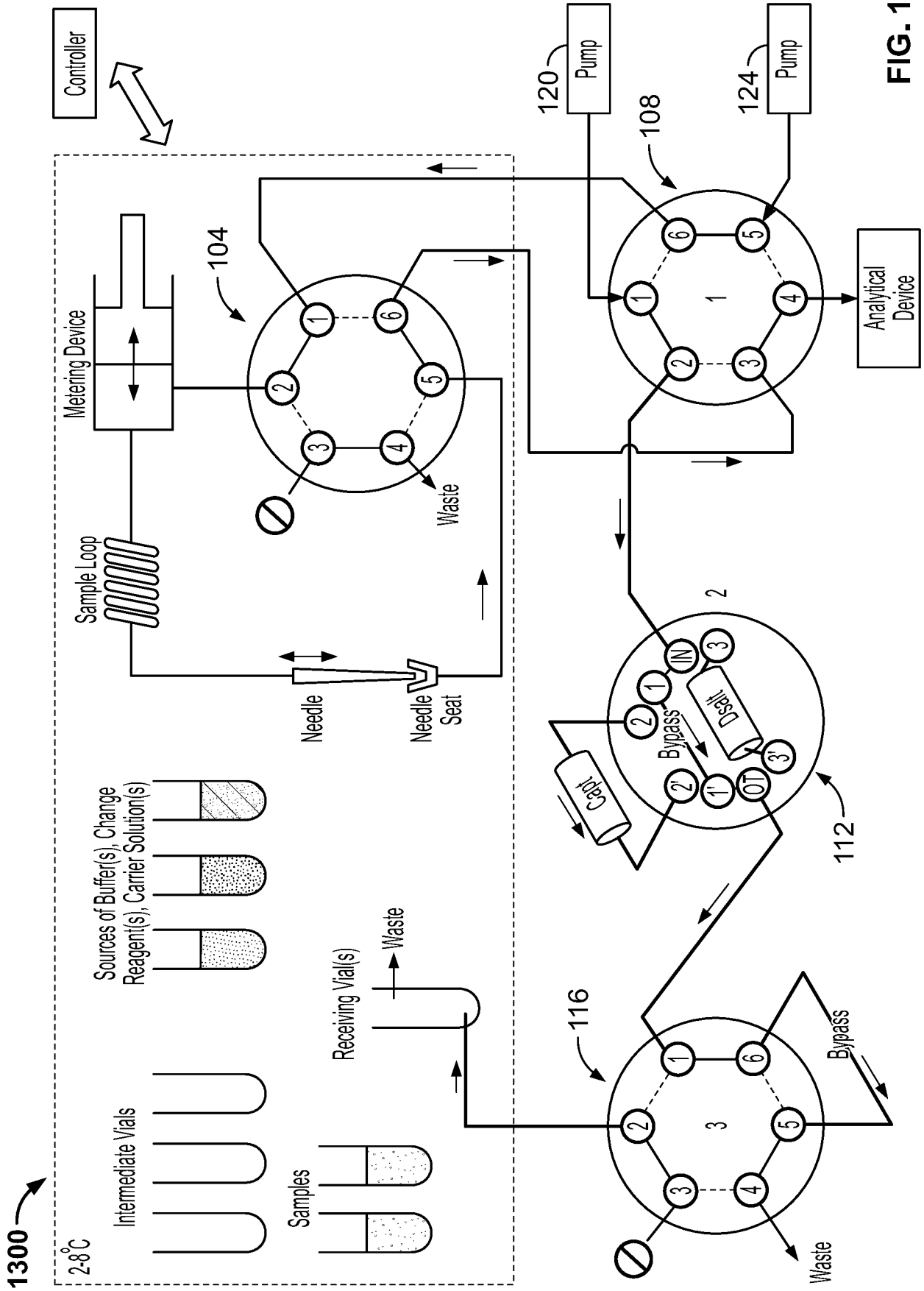

1300
2-8°C
Controller
Metering Device
104
Sample Loop
Needle
Needle Seat
Waste
Sources of Buffer(s), Change Reagent(s), Carrier Solution(s)
Intermediate Vials
Samples
Receiving Vial(s)
Waste
120
Pump
124
Pump
108
1
Analytical Device
2
IN
Bypass
Dsalt
Calt
OT
112
116
3
Bypass
Waste

FIG. 13

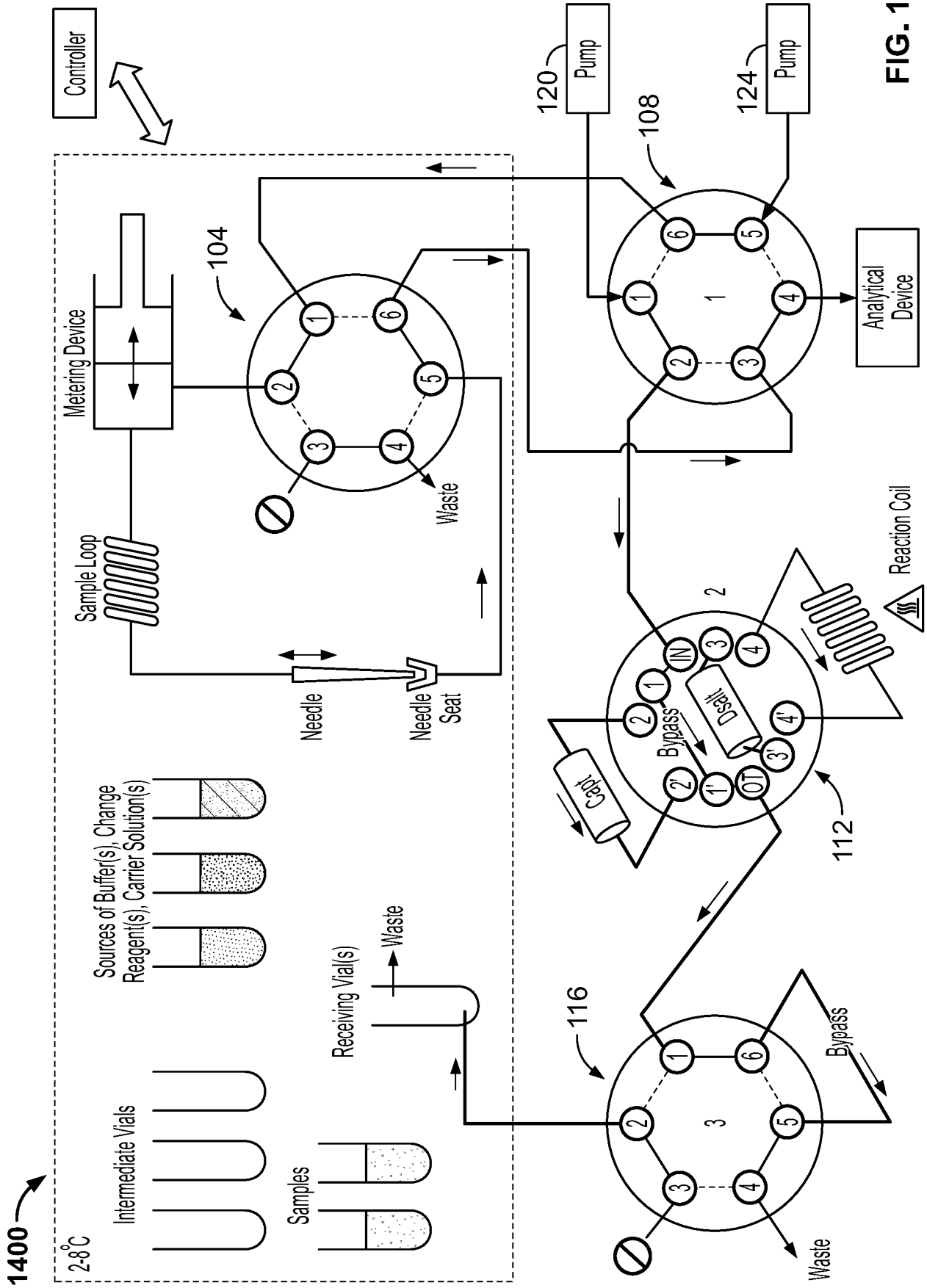

1400
2-8°C
Controller
Metering Device
104
Sample Loop
Needle
Needle Seat
Waste
Sources of Buffer(s), Change Reagent(s), Carrier Solution(s)
Intermediate Vials
Samples
Receiving Vial(s)
Waste
120
Pump
124
Pump
108
Analytical Device
Reaction Coil
Bypass
Dsalt
Calt
112
116
Bypass
Waste

FIG. 14

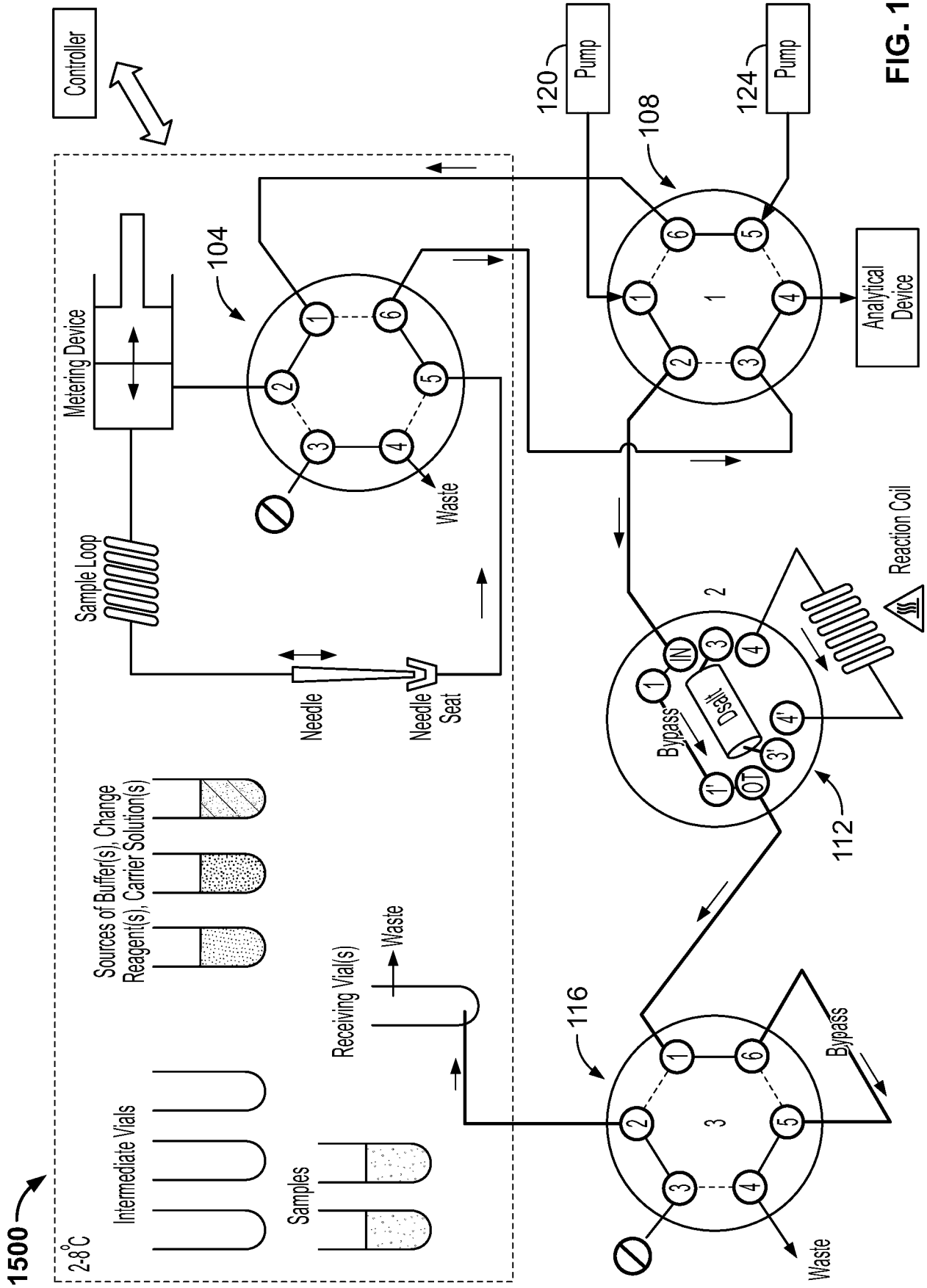

Controller
120
Pump
124
Pump
108
104
Metering Device
Analytical Device
Sample Loop
Waste
Needle
Needle Seat
Reaction Coil
Bypass
Disk
IN
OT
112
Sources of Buffer(s), Change Reagent(s), Carrier Solution(s)
Receiving Vial(s)
Waste
116
Bypass
Intermediate Vials
Samples
Waste
1500
2-8°C

FIG. 15

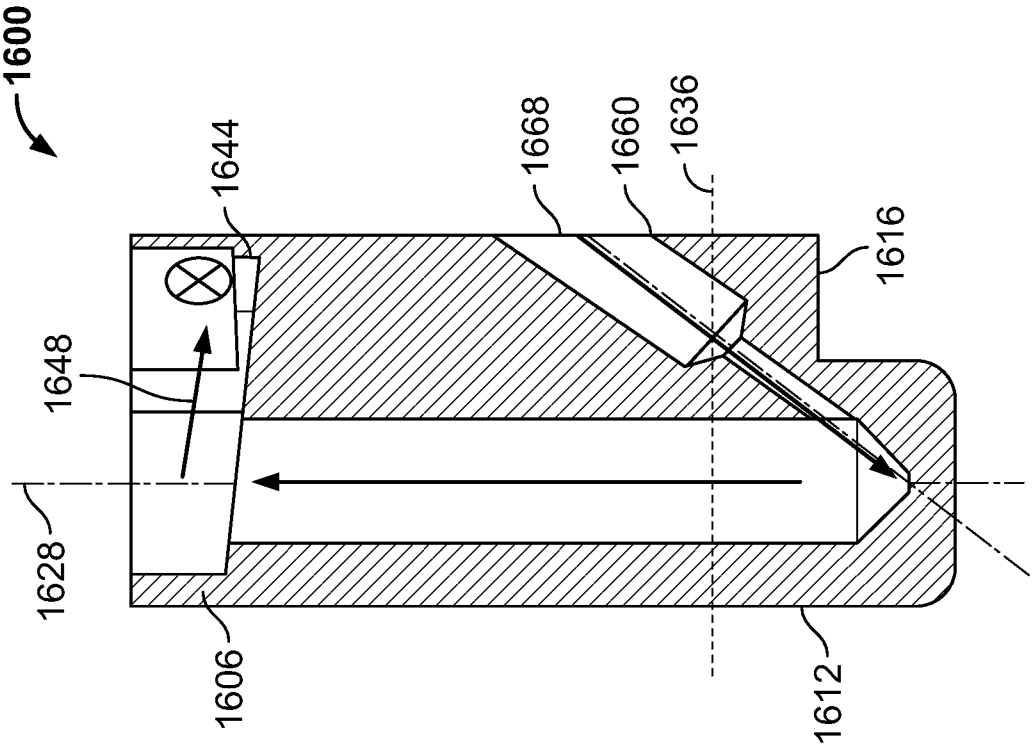

1600
1644
1668
1660
1636
1616
1648
1628
1606
1612

FIG. 17

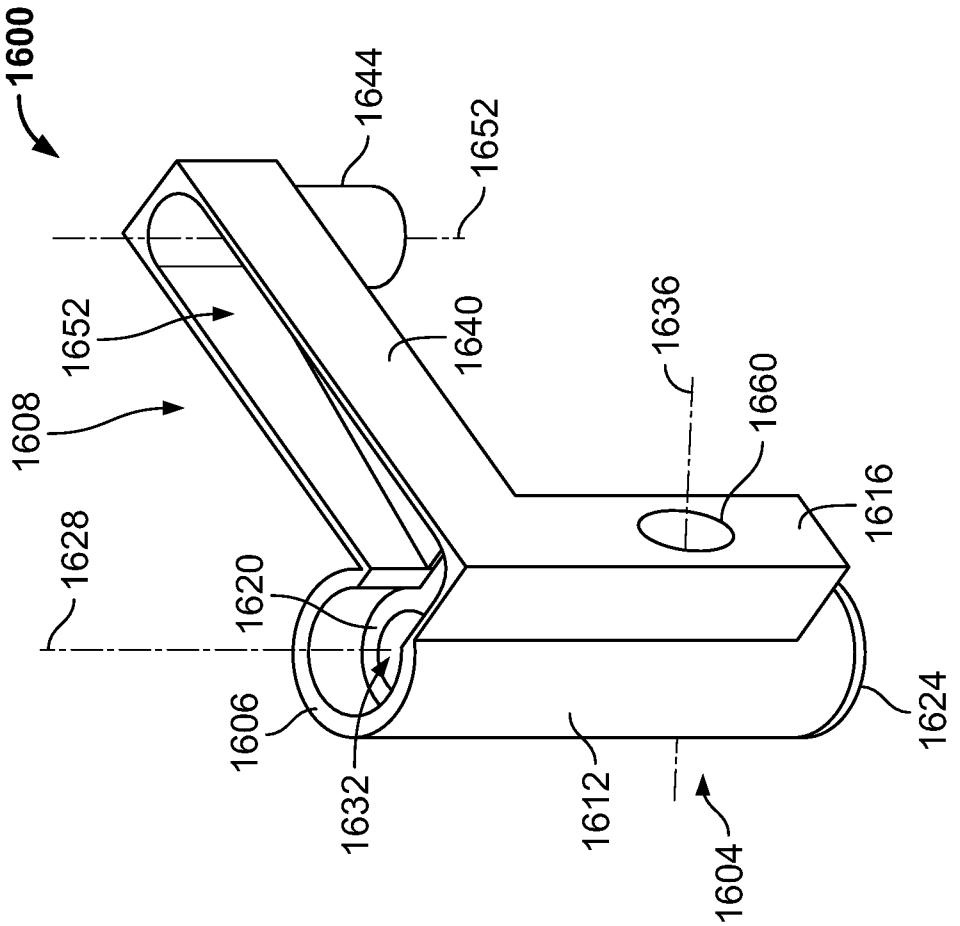

1600
1644
1652
1652
1640
1636
1660
1608
1616
1628
1620
1606
1632
1612
1624
1604

FIG. 16

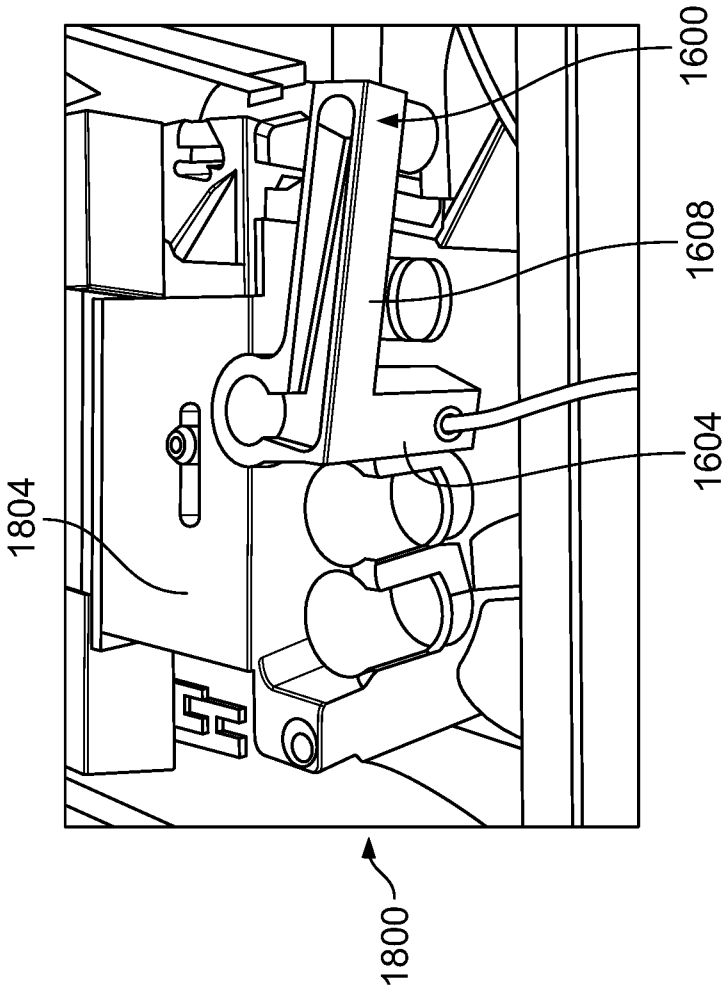


FIG. 19

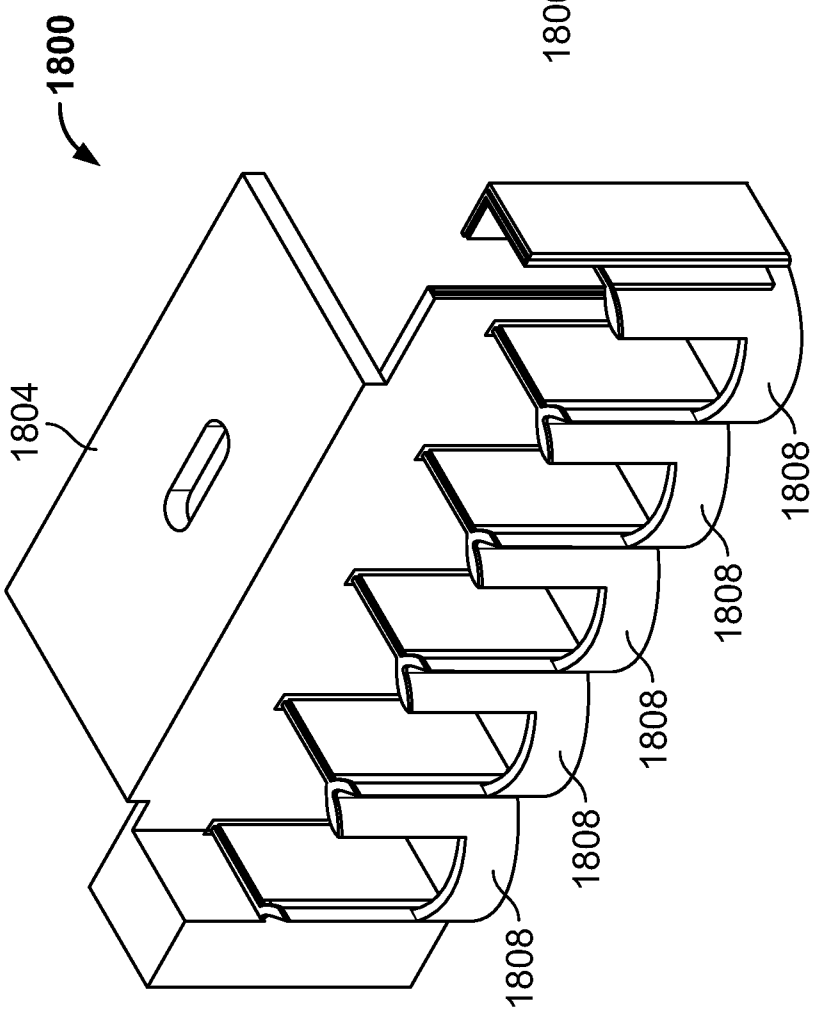


FIG. 18

Molecule	SEC Assay	CEX Assay	rCE Assay
mAb1	✓	✓	✓
mAb2	✓		✓
mAb3	✓	✓	✓
BiTE1	✓	✓	
BiTE2	✓	✓	✓
Bi-specific 1	✓	✓	✓
Fusion 1	✓	✓	✓
Bi-specific 2	✓	✓	✓
xmAB	✓	✓	✓

FIG. 20

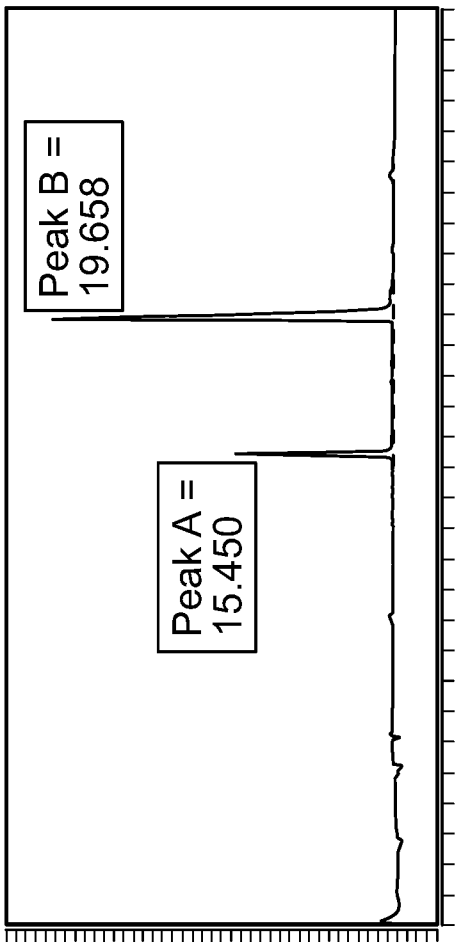


FIG. 21

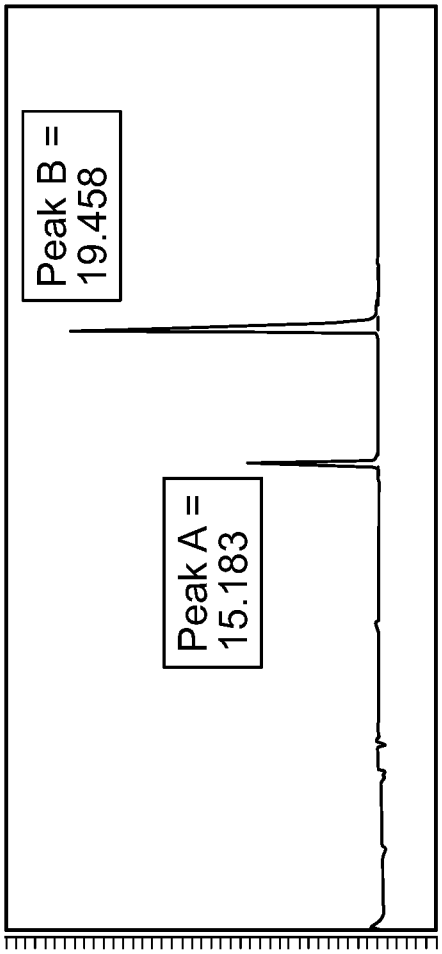


FIG. 22

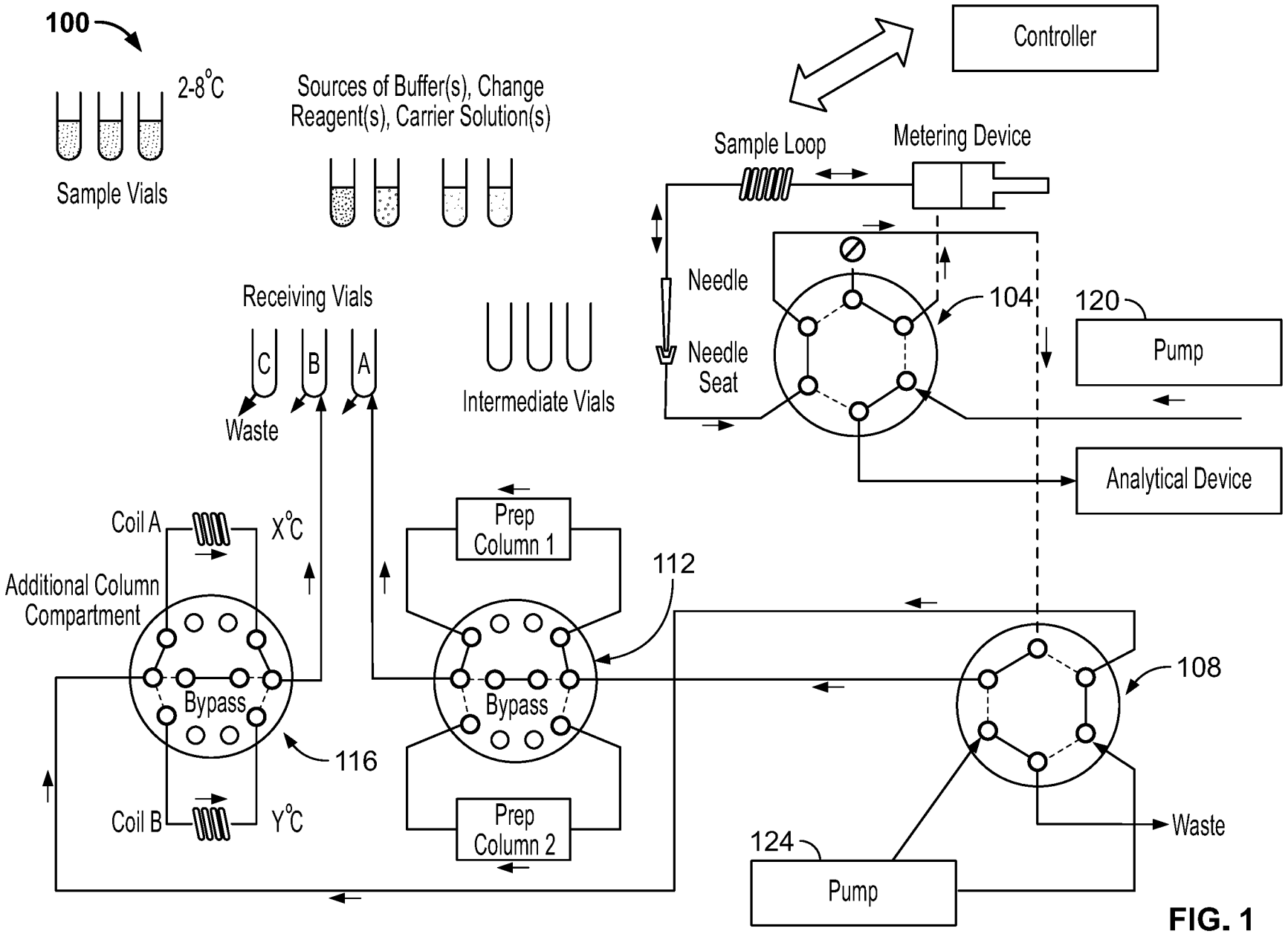

100
2-8°C
Sample Vials
Sources of Buffer(s), Change Reagent(s), Carrier Solution(s)
Controller
Sample Loop
Metering Device
Needle
Needle Seat
104
120
Pump
Receiving Vials
C
B
A
Waste
Intermediate Vials
Analytical Device
Coil A
X°C
Additional Column Compartment
Prep Column 1
112
Bypass
Bypass
108
116
Coil B
Y°C
Prep Column 2
124
Pump
Waste

FIG. 1