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| Dilution Preparation |
| **Purpose** | This procedure provides instructions for DILUTION PREPARATION. The purpose of this procedure is to provide assistance when preparing various dilutions, examples of typical dilution ratios, and to reduce errors related to the dilution of samples. This procedure is provided to Chemistry personnel responsible for preparing dilutions of serum, plasma, urine and other fluids in the Chemistry department.  |
| **Policy Statements** | * This procedure applies to all personnel performing dilutions in chemistry
* When a test result exceeds the assay range or reportable range for a method, a manual dilution may be required.
* Only perform dilutions as outlined in each assay procedure.
* The dilution protocol is verified by checking analyzer results against diluted sample results documented on the [CH 2.03.f1 Dilution Verification Log](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Forms/201922.pdf)
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| **Definitions** | **Dilution**: The process by which the concentration of a given analyte is decreased by the addition of a diluent, often purified water. The dilution is notated as ***Vs:Vt****,* where Vs is Sample volume and Vt is Total volume. For example, dilution of 1mL sample by addition of 2mL diluent would be written as a “1:3 dilution”.**Serial dilution**: A sequential set of dilutions, useful when the sample volume is in short supply. **Dilution Factor**: The inverse ratio of the concentrated sample to the ***total final volume.*** For example, a 1:3 dilution has a dilution factor of 3. |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Documents** |
|  | * Diluent, refer to individual test procedures for required diluent, preparation and stability.
 | * Pipette Tips
* Dilution sample cup or tube
 | * Pipettes: select a pipette that is suitable for the desired volume
 | * [CH 2.03.f1 Dilution Verification Log](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Forms/201922.pdf)
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| **Sample** | Refer to the Specimen Collection Manual for detailed instructions on collection of the specimen.Suitable specimens for dilution include serum, plasma, urine, cerebrospinal fluid (CSF) and other body fluids of adequate volume to aspirate an aliquot without error. See individual analyte procedures for specific test requirements.Insufficient sample could cause incorrect dilution results. |
| **Special Safety Precautions** | Refer to laboratory safety policies and procedures.  |
| **Calibration** | Pipettes used to perform dilutions must have a current calibration (less than 6 months). Calibrated pipettes have a label indicating the last successful calibration date. Glass Class A pipettes do not require calibration, but should be inspected before use for chips, scratches, and scuffs that make the volume line indeterminate. |
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| **Procedures:** |  |  |  |
| **Dilution Technique** | **Step** | Action | **Related Document** |
|  | 1 | MLA style pipettes require use of pre-wetting technique. Pre-wetting the pipette tip involves aspirating the solution and dispensing back into the original container. Then, aspirate a second time and deliver to destination container.  |  |
|  | 2 | Aspirate liquid from the center of the container, inserting pipette tip just below the surface. This will minimize liquid adhering to the outside of the pipette. |  |
|  | 3 | Dispense liquid to the side of the container just above any liquid already inside. This will prevent fluid from the outside of the pipette being transferred to the new container.  |  |
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|  | **Step**Action | **Related Document** |
| **Simple Dilution** | 1 | Only perform manual sample dilutions as outlined in each assay procedure, following guidelines with regard to volumes, diluents, and other limitations. |  |
|  | 2 | Minimally, label the dilution cup or container with accession number, dilution ratio, and the assay to be tested. |  |
|  | 3 | Add diluent to the container labeled for the dilution. Volume and diluent type will be specified by assay procedure. |  |
|  | 4 | Add patient sample to the container labeled for the dilution. Volume will be specified by assay procedure. |  |
|  | 5 | Mix diluent and sample by inversion or with a clean transfer pipette. Do not use the pipette tip used for either the diluent or patient sample – this will introduce additional volume and increase error. |  |
|  | 6 | Follow instrument protocol for programming dilution factor and patient demographics information. |  |
|  | 7 | Test diluted sample according to assay procedure.  |  |
|  | 8 | Record work on the dilution verification log, have a 2nd tech verify work before reporting results. | [CH 2.03.f1 Dilution Verification Log](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Forms/201922.pdf) |
|  | 9 | **If** | **Then** |  |
| Dilution factor was programmed into instrument | DO NOT multiply the analyzer result readout by the Dilution Factor. |
| Dilution factor was not programmed into instrument | Multiply the analyzer result readout by the Dilution Factor. Show calculations on instrument dilution verification log. |
|  | 10 | **If** | **Then** |  |
| Numerical result | Report |
| Result is “less than” | Do not report.  |
| Result is “greater than” | See assay procedure. Either perform higher dilution as allowed by assay procedure, or report as the greater than value. |
|  | 11 | Follow instrument protocol for programming dilution factor and patient demographics information. |  |
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| **Serial Dilution** | **Step** | Action | **Related Document** |
|  | 1 | Follow individual assay procedures for serial dilutions, as applicable. |  |
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| **Interpretation/ Results/Critical Values** | 1. Have all calculations and work checked by a second tech prior to release of results.
2. Final results should not contain error messages.

The smallest dilution with a reportable result will have the least error. |
| **Limitations** | * Only perform manual dilutions as outlined in each assay procedure.
* Do not report values below measuring range (less than values) after a dilution unless otherwise specified.
* The smallest dilution with a reportable result will have the least error.
* Only use non-viscous fluids for dilutions in chemistry assays.
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| **Result Reporting** | Refer to specific assay procedures for instructions on reporting diluted results. |
| **References** | 1. Clinical Chemistry, Principles, Procedures, Correlations, 3rd Edition, Lippincott-Raven, Philadelphia, PA 1996, p. 22-24.
2. Tietz Fundamentals of Clinical Chemistry, 5th Edition, W.B. Saunders Company, 2001, p. 17-18.
3. Dimension® RxL MAX ™ Clinical Chemistry System Operator’s Guide, Dade Behring Inc., Newark, DE, 05/2003, p. A-18
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| **Historical Record** |  |
| **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Linda Lichty | October 16, 2006 | Initial Version |
|  | D. Helfinstine | October 10, 2010 | New Format, added double check that pre/post are within 10%.  |
|  | L. Lichty | April 1, 2011 | Renumbered from CH 6.067 |
|  | L. Lichty | July 2, 2013 | Define minimum sample volume, maximum ratio. |
|  | L. Lichty | 6/1/2015 | Minor updates for Vista |
|  | Kelsi Brown | April 21, 2017 | Biennial Review |
|  | Kelsi Brown | April 22, 2019 | Biennial Review |
|  |  | Matt Johnson | 9/30/2022 | Full revision. Dilution protocols are specified in assay procedures. Technique section added. Clarified definitions section. |