# *Method Comparison Table*

| EPA 365.2 & 365.3 Ascorbic Acid Method | Merck 14XXXP Cell Test |
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| **1.0 Scope and Application**<br><br>Ammonium molybdate and potassium antimonyl tartrate react in (Sulfuric) acid medium with orthophosphate to form a heteropoly acid-phosphomolybdic acid, which is reduced to intensely colored molybdenum blue by ascorbic acid. This color is read at either 690 or 880 nm.<br><br>This Method is applicable for use in the Environmental Protection Agency's (EPA's) survey and monitoring programs under the Clean Water Act.<br><br>The ML is implied as 0.01 mg/L, from the concentration range tested 0.01 – 0.5 mg/L.<br><br>There is no reference to an MDL. | **1.0 Scope and Application**<br><br>This method is intended for the determination of orthophosphoric and total phosphorus on drinking and surface waters, ground water, seawater and industrial wastes water matrices.<br><br>In a solution acidified with sulfuric acid, ortho-phosphate ions react with (Ammonium) molybdate ions to form molybdophosphoric acid.<br><br>Ascorbic acid reduces this to phosphomolybdenum blue (PMB) which is then determined photometrically at or near 690 nm.<br><br>This methods encompasses an extremely broad range spanning from 0.05 – 25 mg P/L (and can be expressed as phosphate concentration in the range of 0.50 – 75 mg/L) Appendix I & II. The MDL has been established at 0.02 mg/L P. The ML for the method is 0.05 mg/L P.<br><br>This method is applicable for use in the Environmental Protection Agency's (EPA's) survey and monitoring programs under the Clean Water Act. |
| **2.0 Summary of Method**<br><br>This method reads orthophosphate. Orthophosphate reacts with ammonium molybdate and potassium antimonyl tartrate in an acid medium to form phosphomolybdic acid. This is reduced to molybdenum blue by ascorbic acid.<br><br>If Total Phosphate is desired a digestion procedure (using ammonium persulfate reagent) must precede the determination. The time for digestion is 30 minutes.<br><br>The color formation takes place after the addition of the ascorbic acid, and the color reaction time is 10 minutes, but measurements of absorbance (at wavelength 690 or 880 nm) should not be taken longer than 30 minutes after reagent addition. | **2.0 Summary of Method**<br><br>This method's reaction is parallel to that sequence of reactions and subsequent color formation identified the Reference Method.<br><br>The digestion time is the same as in the reference method, 30 minutes.<br><br>The reaction time is five minutes versus the ten minutes outlined in the reference method. See appendix III for a diagram outlining the color complex stability through a ten minute period.<br><br>The absorbance of the color complex is determined at the same wavelength (690 nm) as the reference method. |
| **3.0 Definitions**<br><br>See Section 18.0–There are no terms, acronyms, or symbols, which have been defined in this method. | **3.0 Definitions**<br><br>See section 18.0 – This method defines, in great detail, the terms, acronyms, and symbols, which appear in the body of the method. |

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| **4.0 Interferences**<br><br>Arsenates react with the molybdate to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. The interference may be eliminated by reductin arsenic acid to arsenious acid with sodium bisulfite.<br><br>High concentrations of iron cause low recovery of phosphorus, and may be eliminated with the addition of sodium bisulfite. | **4.0 Interferences**<br><br>This Method is subject to the same interferences as found in the Reference Method (See Appendix I).<br><br>Color and suspended matter, may interfere with the photometric measurement. To counter this potential positive interference, the sample may be filtered (after digestion) or prepare a sample blank for correction of the interferences. |
| **5.0 Safety**<br><br>The reference method does not define potential health risks associated with the use of the chemicals in this method. | **5.0 Safety**<br><br>This method employs the use of test cells which reduces the volume/mass of reagent chemicals. As with any analytical procedure, the analyst is cautioned to become familiar with the potential health hazards described in the reference Material Safety Data Sheet (MSDS) records.<br><br>Spectroquant® Phosphate Cell Tests, 14729 and 14543 are also clearly labeled. This increases likelihood of user safety pursuant to analysts' ready ability to recognize presence of reagents peculiar to the protocol being implemented.<br><br>The analyst is also cautioned to use extreme care when handling heated cells, and when working near the thermoreactor. |
| **6.0 Equipment and Supplies**<br><br>This method employs standard laboratory glassware for sampling (1L glass or plastic bottle).<br><br>Standard glassware for sample handling include pipettes, blender, culture tubes (16 x 100-mm, 20 x 150-mm, and 25 x 150-mm).<br><br>As this method is extremely sensitive, the absence of interferences from residual detergents on glassware is stressed. A procedure for glassware preparation is outlined, and it is suggested that the analyst dedicate glassware for use only on this parameter, to avoid cross contamination.<br><br>A spectrophotometer is used at either 690 nm or 880 nm. | **6.0 Equipment and Supplies**<br><br>This method employs all equipment and supplies, which are specified in EPA methods 365.2 and 365.3. The photometric determination is accomplished using a Merck Spectroquant® system photometer or any other photometric device capable of measuring absorbance at or near 690 nm.<br><br>This method employs standard laboratory glassware for sampling (1L glass or plastic bottle).<br><br>Standard glassware for sample handling include pipettes and volumetric flasks. The risk of contamination from phosphate detergents is present to a lesser extent than in the reference method, as the cells with reagents are self-contained, and are not reusable. The reagent additions are accomplished using a dosing system, which does not require invasive handling or measurements.<br><br>Spectroquant® Phosphate Cell Test 14543 and 14729. These products contain the 16 x 100 mm cells with pre- |

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| | measured reagents. The cells may be used for total P digestion (if required) and also for photometric determination.<br><br>A Spectroquant® Thermoreactor is used to accomplish the 30 minute digestion of the total phosphorus samples.<br><br>The photometric determination is accomplished using a photometer or spectrophotometer. A description of the Merck Spectroquant® system photometer is listed below.<br><br>***Merck Spectroquant® system photometer***<br>*Phosphate cell identification* – The Merck Spectroquant® system photometer is equipped with a Sample ID system. Each Spectroquant® Phosphate Cell Test is bar coded, and when placed correctly in the cell compartment, the instrument recognizes the cell, and sets the instrument to the proper measuring parameters (i.e. item number, test range, cell format, wavelength and calibration data).<br><br>These instruments can also store the sample information within its data files, for printing, downloading to alternate data storage location, or for easy retrieval.<br><br>*Calibration* - The Merck Spectroquant® system photometers have been factory calibrated, and the readings obtained from the sample cells are automatically expressed as the concentration of the analyte in mg/L. The calibration curve can be verified, and the data from this verification can be stored, modified or re-entered at any time.<br><br>The user cannot change the factory program setting. Instead, when the manufacturer generates new calibration data, they supply a new memo microchip (transponder) to the user for updating the system.<br><br>***Other Photometric Devices***<br>The use of other photometric equipment, which may be substituted for use with this method, is consistent with the reference method specifications. |
| **7.0 Reagents and Standard**<br><br>This method incorporates the following chemicals which are purchased and/or prepared by the laboratory analyst:<br><br>a. Sulfuric Acid, $H_2SO_4$, CAS 7664-93-9<br><br>5 N: Dilute 70 ml concentrated $H_2SO_4$ to 500 ml with distilled water. | **7.0 Reagents and Standards**<br><br>The pre-measured reagents reduce the risk of error in preparation of the chemical reagents.<br><br>a. Sulfuric Acid CAS 7664-93-9 |

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| b. Potassium antimonyl tartrate solution 28300-74-5<br><br>Dissolve 1.3715 grams $K(SbO)C_4H_4O_6$-1/2$H_2O$ in 400 ml distilled water in a 500ml volumetric flask and dilute to volume. Store in a glass stoppered bottle. | b. Potassium antimony (III) oxiditartrate hemihydrate CAS 28300-74-5 |
| c. Ammonium molybdate solution CAS 12054-85-2<br><br>Dissolve 20 grams $(NH_4)_6Mo_7O_{24}$-4$H_2O$ in 500 ml distilled water. Store in a glass-stoppered bottle. | c. Ammoniumheptamolybdate Tetrahydrate CAS 12054-85-2 |
| d. Ascorbic Acid, 0.1 M ACS 50-81-7<br><br>Dissolve 1.76 grams ascorbic acid in 100 ml distilled water. The solution is stable for about 1 week at 4 Degrees C. | d. Ascorbic Acid CAS 50-81-7 |
| e. Combined reagent: Mix the above reagents in the following proportions for 100ml of the combined reagent: 50 ml 5 N $H_2SO_4$, 5 ml potassium antimonyl tartrate solution, 15 ml ammonium molybdate solution, and 30 ml ascorbic acid solution. Mix after addition of each reagent. Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent s stable for 4 hours.<br>f. Standard phosphate solution: Dissolve in distilled water 219.5 mg anhydrous $KH_2PO_4$ and dilute to 1000ml: 1.00ml = 50 ug $PO_4^{3-}$-P.<br>g. Standard phosphate solution: Dilute 50.0 ml stock phosphate solution to 1000ml with distilled water; 1.00ml = 2.50 ug $PO_4^{3-}$-P.<br>h. 11 N $H_2SO_4$; add 300 ml concentrated $H_2SO_4$ to 600 ml of deionized water. Cool to room temperature and dilute to 1 Liter.<br>i. Ammonium persulfate, $(NH_4)_2S_2O_8$ | e. Standard Phosphate solution (See Section 7): Phosphate Stock solution (1000 mg/L) diluted 10:100 to create working std solution (100 mg P/L) |
| **8.0 Sample Collection, Preservation, and Storage**<br><br>If Phosphorous forms are to be differentiated, filter sample immediately after collection. Preserve by freezing at or below –10 Degrees Centigrade. Add 40 mg HgCl2/L to the samples, especially when they are to be stored for long periods. Caution: $HgCl_2$ is a hazardous substance; take appropriate precautions in disposal.<br><br>Do not add either acid or $CHCl_3$ as a preservative when phosphorous forms are to be determined. If total | **8.0 Sample Collection, Preservation, and Storage**<br><br>The sampling is performed in accordance with Standard Methods. There are no differences in the way the samples are collected. If analysis is not effected immediately, preservation and storage of samples in this method is identical to these procedures in the Reference Method.<br><br>This method does not propose the addition of $HgCl_2$ to the samples, as this is an unnecessary addition of a hazardous chemical. The analysis of the samples can usually be |

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| phosphorous alone is to be determined, add 1 ml concentrated HCl/L or freeze without any additions.<br><br>Do not store samples containing low concentrations of phosphorous in plastic bottles unless kept in a frozen state because phosphates may be adsorbed onto the walls of plastic bottles.<br><br>Rinse all glass containers with hot dilute HCl, then rinse several times in distilled water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis. | performed within the proper holding times.<br><br>Rinse all glass containers with hot dilute HCl, then rinse several times in distilled water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis. |
| **9.0 Quality Control**<br><br>There are no quality control guidelines incorporated into the body of this method. | **9.0 Quality Control**<br><br>This method includes guidelines for initial demonstration of laboratory capability, quality control and quality assurance measurements.<br><br>Initial demonstration of performance of the method is required. After initial performance has been established, the analyst is required to provide proof of continued performance through the analysis of ongoing precision and recovery standards. These are tested in conjunction with the entire analytical quality control batch (for up to 20 samples), which include: (1) laboratory blank, (1) laboratory control sample (OPR), (1) Spiked sample (MS), and (1) spiked sample duplicate (MSD). |
| **10.0 Calibration and Standardization**<br><br>The spectrophotometer is calibrated using a blank and at least five (5) P standards covering the range of the test 0.01 – 0.50 mg/L. The absorbances of the standards is plotted against the concentration. Absorbance readings from samples are plotted against this curve to obtain a concentration value.<br><br>The calibration curve should be re-run if the linearity of the curve varies more than 5%, or with each new lot of reagents. | **10.0 Calibration and Standardization**<br><br>Determine which range is applicable for determination of the amount of phosphate believed to be present in the sample. Begin with a stock Phosphate Solution (1000 mg/L) and prepare a series of standards spanning both test ranges.<br><br>The calibration curve should contain a minimum of five calibration points plus a reagent blank with which to zero the photometric instrument.<br><br>Other points can be selected to cover the range, or inclined to populate intervals along the range whereupon the samples' concentrations are anticipated to absorb.<br><br>***Merck Spectroquant® system photometer***<br>The instruments are factory calibrated with standard reference material, and the products are shipped with Lot Certificates for calibration (Appendix IV). |

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| | ***Other Photometric Devices***<br>Calibration for absorbance measurements using these instruments or other photometric devices is performed as described in the reference method. |
| **11.0 Procedure**<br><br>For total phosphorus digestion, measure 50 ml of sample into a 125 ml Erlenmeyer flask. Add ammonium persulfate. Boil gently to digest for approximately 30 minutes. The sample is then cooled, pH adjusted and if the sample is not clear, add 2-3 drops of acid, and filter to remove turbidity. Proceed to measurement procedure.<br><br>pH adjust all other samples to 7.0 ± 0.2 before proceeding.<br><br>To 50 ml of sample, add molybdenum antimony potassium tartrate and ascorbic acid.<br><br>After at least 10 min but no more than 30 min, measure absorbance of each sample at 690 or 880 nm, using reagent blank as the reference solution.<br><br>Correction for turbidity or interfering color: Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract blank absorbance from absorbance of each sample. | **11.0 Procedure**<br><br>pH must be within 0-10. Adjust, if necessary, by adding a drop of (0.5%) aqueous solution phenolphthalein indicator. Add 5 N H2SO4 solution dropwise to discharge the color.<br><br>Pipette an aliquot (depending upon the range desired) of sample (pH 0-10) into the appropriate Phosphate Cell Test. Mix well.<br><br>For Total Phosphate analyses, measure one dose of P-1K (Potassium peroxidisulfate) into the cell, mix well, and heat in the thermoreactor for 30 minutes. After the cell is cooled proceed as in ortho-phosphate analysis.<br><br>To each cell add five drops of Reagent P-2K (analogous to molybdenum antimony potassium tartrate reagent is reference method).<br><br>Add one metering dose of P-3K (ascorbic acid) to each cell. Mix well, and allow for a five minute reaction.<br><br>After the five minute reaction time, wipe clean the test tube. Place the tube into the cell compartment of the photometer, and measure the absorbance at or near 690 nm. Read absorbance photometrically within 30 minutes.<br><br>Samples may be filtered after digestion to avoid false positive readings on the samples. A sample blank can also be prepared with all reagents except ascorbic acid. The absorbance result of this blank may be deleted from the sample absorbance readings. |
| **12.0 Data Analysis and Calculations**<br><br>The reference method addresses significant digits. Results are to be reported to three significant digits, and the Minimum Level (ML) for the method is 0.01 mg/L. | **12.0 Data Analysis and Calculations**<br><br>***Merck Spectroquant® system photometer***<br>Results with a Merck Spectroquant® system photometer are displayed as concentration (mg/L).<br><br>***Other Photometers***<br>Measurement of samples with equivalent photometric equipment allows for absorbance values to be plotted from calibration curves.<br><br>This method defines the calculation for dilution factor correction in event that samples read outside the calibration range of the test. |

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| | The Minimum Level (ML) for this method is 0.05 mg/L. If a result is obtained which is lower than this ML, report the result as less than the ML (<0.05 mg/L). Report results to three significant digits. |
| **13.0 Method Performance**<br><br>Section 10 of the EPA method summarizes precision and accuracy data obtained for organic and orthophosphate from approximately 20 laboratories. | **13.0 Method Performance**<br><br>Reference method, this method incorporates the same chemical components, in the same proportions, the same digestion time and temperature, and is determined similarly via photometric measurement. The precision and bias stated in the reference method are achievable by this method. |
| **14.0 Pollution Prevention**<br><br>The reference method does not discuss pollution prevention. | **14.0 Pollution Prevention**<br><br>Packaging and use of pre-measured, and manipulated reagents for Spectroquant® Phosphate Cell Tests are designed to minimize risks of spillage, and to reduce the amounts of the chemicals used.<br><br>The laboratory is reminded to properly manage these reagents in the laboratory to reduce any threat to the environment.<br><br>General practices, such as ordering of supplies, can seriously impact the amount of materials which require disposal in the laboratory. It is suggested that the laboratory only order supplies as demand dictates, to minimize expired materials requiring disposal. |
| **15.0 Waste Management**<br><br>The reference method does not discuss waste management. | **15.0 Waste Management**<br><br>Referenced in section 15.3 are two waste management documents for further information on this subject.<br><br>In using this method, the laboratory must comply with all federal, state, and local regulations governing waste management. |
| **16.0 References**<br><br>The EPA method lists four reference method documents, including Standard Methods. | **16.0 References**<br><br>This method incorporates the two reference methods, the product insert sheets, instrument manuals, a calibration method, and the ATP document as reference documents. |
| **17.0 Tables, Diagrams, Flowcharts, and Validation Data**<br><br>This method incorporates a flow chart for the scheme for the differentiation of phosphorus forms. Also, there are two tables summarizing precision and accuracy data | **17.0 Tables, Diagrams, Flowcharts, and Validation Data**<br><br>There are three tables in this method: |

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| obtained in multi-laboratory studies. | ***Table 1***<br><br>This table summarizes the test ranges, product numbers, and sample volumes.<br><br>***Table 2***<br><br>This table defines the procedure for preparing standard solutions for instrument calibration at each of the ranges.<br><br>***Table 3***<br><br>This table lists the standardized QC acceptance criteria, which are cited in the ATP protocol, Appendix IF. A laboratory must achieve these criteria before conducting analyses on samples with this method. |
| **18.0 Glossary**<br><br>The reference method defines the forms of phosphorus, which may be analyzed and quantified by this method.<br><br>No other definitions are listed in the method. | **18.0 Glossary**<br><br>See Section 3.0<br><br>The glossary defines terminology used in the body of method. Much of the terms defined are specific to the quality control section of the method. These terms are not used, or defined, in the reference method.<br><br>This section clearly defines the nomenclature of the products being proposed for use. |