Enterovirus RNA Detection by Rapid Polymerase Chain Reaction (PCR)

Clinical
- The Enterovirus group includes the polioviruses (3 types), echoviruses (32 types), coxsackievirus A (23 types) and coxsackievirus B (6 types). These viruses cause systemic infections involving many organ systems. Collectively, they are the most common cause of upper respiratory tract disease in children. In addition, the enteroviruses are the most common cause of central nervous system (CNS) disease; they account for almost all viruses recovered from spinal fluid. Differentiation of enteroviruses from other viruses and bacteria causing CNS disease is important for the appropriate medical management of these patients.

Traditional cell culture methods require, on average, 6 days for enterovirus detection. Real-time polymerase chain reaction (PCR) allows same-day detection.

Useful For
- Rapid diagnosis of CNS infection caused by the enteroviruses

Interpretation
- Detection of enterovirus nucleic acid by PCR is the most sensitive diagnostic method for the diagnosis of CNS infection caused by these viruses. The assay detects greater than 10 genomic equivalents of the virus.

Cautions
- A negative result does not rule out the possibility of enterovirus infection in the CNS.

Supportive Data
- Specificity: 50 spinal fluid specimens submitted to the Division of Clinical Chemistry (but not the Division of Clinical Microbiology) were negative for enterovirus nucleic acid by LightCycler PCR. In addition, the enterovirus LightCycler PCR was specific in that the test was negative with target nucleic acid of other RNA-containing viruses (influenza virus, types A and B; respiratory synerial virus and parainfluenza virus) and DNA-containing viruses (herpes simplex, Epstein-Barr virus, varicella-zoster virus, and cytomegalovirus).

Sensitivity: we compared the generic detection of enteroviruses from spinal fluid by conventional tube cell culture (MCR-5) and by LightCycler PCR. Of 715 specimens tested, enteroviruses were detected in 65 (9%) by conventional cell culture and 82 (11%) by LightCycler PCR. Twenty-two of 82 (27%) were exclusively positive by PCR, whereas, only 5 of 65 (8%) were exclusively positive by conventional cell cultures.

References
Method

LightCycler PCR has been optimized to detect common conserved sequences in the 5’ nontranslated region (NTR) consisting of 711 to 822 nucleotides of enterovirus genotypes.

Viral nucleic acid is extracted by the MagNA Pure automated instrument (Roche Applied Science) from spinal fluid specimens. Primers directed to the 5’ NTR of the enterovirus genome amplify a 193 bp product. For the test enterovirus genomic RNA is transcribed to cDNA. The LightCycler instrument (Roche Applied Science) amplifies and monitors the development of target nucleic acid sequences after the annealing step during PCR cycling by fluorescence assay. This automated PCR system can rapidly (30-40 minutes) detect amplicon development through stringent air-controlled temperature cycling and capillary cuvettes. The detection of amplified products is based on the fluorescence resonance energy transfer (FRET) principle. For FRET product detection, a hybridization probe with a donor fluorophore, fluorescein, on the 3’-end is excited by an external light source and emits light that is absorbed by a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5’-end. The acceptor fluorophore then emits a light of a different wavelength that can be measured with a signal that is proportional to the amount of specific PCR product. Analysis of the PCR amplification and probe melting curves is accomplished through the use of LightCycler software. (Cockerill FR III, Uhl JR; Applications and challenges of real-time PCR for the clinical microbiology laboratory. In Rapid Cycle Real-Time PCR Methods and Applications. Edited by U Reischl, C Wittwer, and F Cockerill. Berlin, Germany, Springer; 2002, pp 3-30).

Specimen Required: 1.0 mL of spinal fluid. Send specimen refrigerated in a screw-capped, sterile vial. Maintain sterility and forward promptly. Specimens grossly contaminated with blood may inhibit the PCR and produce false-negative results. The high sensitivity of amplification by PCR requires the specimen be processed in an environment in which contamination of the specimen by enterovirus RNA is not likely.

**NOTE:** Please complete a “Microbiology Request Form” (Supply T244) or a “MayoConnect Additional Test Information Form” (Supply T357) and forward it with the specimen.

Reference Values: Negative
Positive results will be reported as enterovirus RNA detected.

**NOTE:** Assay performed using analyte-specific reagent. See “Analyte-Specific Reagents (ASR) – Mayo Medical Laboratories” in Special Instructions.

Analytic Time: 1 day/same day
Days Set Up: Monday through Saturday
Fee: $197.90
CPT Code: 87798
Infection with JC virus (JCV) (polyomavirus group) occurs early in life; about one-half of adults have serologic evidence of past infection with this virus. Infection with JCV is asymptomatic in immunocompetent individuals. JCV is considered the etiologic agent of progressive multifocal leukoencephalopathy (PML), a demyelinating disease that occurs mostly in immunocomprised adults. PML occurs in approximately 5% of all AIDS patients. The virus typically infects the oligodendrocyte in the white matter of the brain, which results in focal or multifocal plaques of demyelination. Another closely related virus, BK virus (BKV) (75% DNA homology), is not a recognized cause of central nervous system (CNS) disease; however, this virus is associated with kidney infections, and in renal and bone marrow transplant patients who develop ureteral stenosis, hemorrhagic cystitis and nephropathy.

Detection of JCV DNA in spinal fluid supports the clinical diagnosis of CNS due to JCV. The assay detects greater than 10 genomic equivalents of the virus.

A negative result does not rule out the possibility of JCV infection in the CNS.

LightCycler PCR assay was compared with the existing conventional nucleic acid amplification and product detection (gel electrophoresis and Southern blot) methods. For the study, 78 archived spinal fluid specimens (previously tested by conventional PCR) were tested by both methods. Thirty-two spinal fluid specimens were positive and 39 specimens were negative by both LightCycler PCR and conventional PCR. Six additional specimens were positive by the LightCycler PCR assay (3 JCV; 3 BKV); 1 specimen was positive by conventional PCR that was not detected by LightCycler PCR. Specificity: 50 spinal fluid specimens submitted to the Division of Clinical Chemistry (but not the Division of Clinical Microbiology) were negative to JCV DNA by LightCycler PCR. In addition, the JCV LightCycler PCR was specific in that the test was negative for target nucleic acid of herpesviruses (cytomegalovirus, herpes simplex virus, Epstein-Barr virus, and varicella-zoster).

References
Viral nucleic acid is extracted by the MagNA Pure automated instrument (Roche Applied Science) from spinal fluid specimens. Primers directed to the VP2 gene, which is conserved between JCV and BKV, produce a 131 bp amplicon. The LightCycler instrument (Roche Applied Science) amplifies and monitors the development of target nucleic acid sequences after the annealing step during PCR cycling. This automated PCR system can rapidly (30-40 minutes) detect amplicon development through stringent air-controlled temperature cycling and capillary cuvettes. The detection of amplified products is based on the fluorescence resonance energy transfer (FRET) principle. For FRET product detection, a hybridization probe with a donor fluorophore, fluorescein, on the 3’-end is excited by an external light source and emits light that is absorbed by a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5’-end. The acceptor fluorophore then emits a light of a different wavelength that can be measured with a signal that is proportional to the amount of specific PCR product. LightCycler hybridization probes are designed for exact homology to JCV; however, each of the 2 probes had a single base mismatch compared to the BKV sequence. Melting curve feature of the LightCycler assay confirms the specificity of JCV amplified product.(Whiley D, Mackay IM, Sloots TP: Detection and differentiation of human polyomaviruses JC and BK by LightCycler PCR. J Clin Microbiol 2001;39:4357-4361).

**Specimen Required:**
1.0 mL of spinal fluid. Send specimen refrigerated in a screw-capped, sterile vial. Maintain sterility and forward promptly. Specimens grossly contaminated with blood may inhibit the PCR and produce false-negative results. The high sensitivity of amplification by PCR requires the specimen be processed in an environment in which contamination of the specimen by JC or BK virus DNA is not likely.

**NOTE:** Please complete a “Microbiology Request Form” (Supply T244) or a “MayoConnect Additional Test Information Form” (Supply T357) and forward it with the specimen.

**Reference Values:**
Negative
Positive results will be reported as JC or BK Virus DNA detected.

**NOTE:** Assay performed using analyte-specific reagent. See "Analyte-Specific Reagents (ASR) – Mayo Medical Laboratories" in Special Instructions.
Cobalt is a silvery, bluish-white, odorless, and magnetic metal. While rare, it is widely distributed in the environment. Cobalt is an essential element, although cobalt deficiency has not been reported in humans. It is an essential cofactor in vitamin B-12.

Cobalt is used in the manufacture of hard alloys with high melting points and resistance to oxidation. Cobalt salts also are used in the glass and pigment industry. Several years ago, cobalt salts were sometimes used as foam stabilizers in the brewing industry; this practice was banned because of the cardiovascular diseases it induced. The radioactive isotope of cobalt, $^{60}$Co, is used as a gamma emitter in experimental biology, cancer therapy, and industrial radiography.

Cobalt is not highly toxic but large enough doses will produce negative clinical manifestations. Acute symptoms are pulmonary edema, allergy, nausea, vomiting, hemorrhage, and renal failure. Chronic symptoms include pulmonary syndrome, skin disorders, and thyroid abnormalities. The inhalation of dust during machining of cobalt alloyed metals can lead to interstitial lung disease. Improperly handled, $^{60}$Co can cause radiation poisoning from exposure to gamma radiation.

Detecting cobalt toxicity
Concentrations ≥2.0 µg/24-hour specimen indicate possible environmental or occupational exposure. Toxic concentrations are ≥5.0 µg/24-hour specimen. These reference intervals are based on unpublished internal observations.

There are no OSHA blood or urine criteria for occupational exposure to cobalt.

Cautions
- This test should not be ordered to assess vitamin B-12 activity.
- Specimen collection procedures for cobalt require special specimen collection tubes, rigorous attention to ultraclean specimen collection and handling procedures, and analysis in an ultraclean facility. Unless all of these precautions are taken, elevated serum cobalt results may be an incidental and misleading finding.

References
Test Title: Cobalt, Urine #80083

**Method**

Aqueous acidic calibrating standards with urine matrix are diluted with an aqueous acidic diluent containing gallium as an internal standard. Specimens are then aspirated into a pneumatic nebulizer and the resulting aerosol is directed to the hot plasma discharge by a flow of argon. In the annular plasma, the aerosol is vaporized, atomized, and then ionized. The ionized gasses plus neutral species formed in the annular plasma space are aspirated from the plasma through an orifice into a quadrupole mass spectrometer. The mass range from 1 to 263 amu is rapidly scanned multiple times and ion counts tabulated for mass 59 (cobalt) and mass 69 (gallium). Instrument response is defined by the linear relationship of analyte concentration versus ion count ratio (analyte ion count/internal standard ion count). Analyte concentrations are derived by reading the ion count ratio for each mass of interest and determining the concentration from the response line. (Houk R, Fassel VA, Flesch GD, et al: Inductively Coupled Argon Plasma as an Ion Source for Mass Spectrometric Determination of Trace Elements. Anal Chem1980;53:2283-2289)

**Specimen Required:**
1. 10 mL from a 24-hour urine collection. No preservative. See “Urine Preservatives” in Special Instructions for multiple collections.
2. Collect in clean, plastic urine container(s) with no metal cap(s) or glued insert(s).
3. Send specimen in a plastic, 13-mL urine tube or in another clean, plastic aliquot container with no metal cap or glued insert. See “Metals Analysis-Collection and Transport” in Special Instructions in “2002 Test Catalog.”
4. Refrigerate specimen within 4 hours of completion of 24-hour collection. Send specimen refrigerated.

**NOTE:** 24 HOUR VOLUME IS REQUIRED ON REQUEST FORM FOR PROCESSING. Gadolinium imaging media (such as in the contrast agent Omniscan) should not be administered within 48 hours of specimen collection.

**Urine Preservative Collection Options**

**IMPORTANT NOTE:** The addition of preservative or application of temperature controls must occur within 4 hours of completion of the collection OR an acceptable preservative must be added at the START of the collection. Urine tests which require that the preservative be added at the START of the collection are noted by an *(asterisk)*.

| Ambient: | Yes |
| Refrigerate: | Preferred |
| Frozen: | No |
| 6N HCl: | Yes |
| Acetic Acid 50%: | No |
| Na2CO3: | No |
| Toluene: | No |
| HNO3: | No |
| Boric: | No |
| Thymol: | No |

**Reference Values:** <2.0 µg/24 hr specimen

Reference interval does not apply to random specimens.

**Analytic Time:** Same day/1 day

**Days Set Up:** Tuesday

**Fee:** $98.10

**CPT Code:** 83789
Cobalt is a silvery, bluish-white, odorless, and magnetic metal. While rare, it is widely distributed in the environment. Cobalt is an essential element, although cobalt deficiency has not been reported in humans. It is an essential cofactor in vitamin B-12.

Cobalt is used in the manufacture of hard alloys with high melting points and resistance to oxidation. Cobalt salts are also used in the glass and pigment industry. Several years ago, cobalt salts were sometimes used as foam stabilizers in the brewing industry; this practice was banned because of the cardiovascular diseases it induced. The radioactive isotope of cobalt, 60Co, is used as a gamma emitter in experimental biology, cancer therapy, and industrial radiography.

Cobalt is not highly toxic but large enough doses will produce negative clinical manifestations. Acute symptoms are pulmonary edema, allergy, nausea, vomiting, hemorrhage, and renal failure. Chronic symptoms include pulmonary syndrome, skin disorders, and thyroid abnormalities. The inhalation of dust during machining of cobalt alloyed metals can lead to interstitial lung disease. Improperly handled, 60Co can cause radiation poisoning from exposure to gamma radiation.

Detecting cobalt toxicity

Concentrations ≥1.0 ng/mL indicate possible environmental or occupational exposure. Toxic concentrations are ≥5.0 ng/mL. These reference intervals are based on unpublished internal observations.

There are no OSHA blood or urine criteria for occupational exposure to cobalt.

Method

Aqueous acidic calibrating standards with serum matrix are diluted with an aqueous acidic diluent containing gallium as an internal standard. Specimens are then aspirated into a pneumatic nebulizer and the resulting aerosol is directed to the hot plasma discharge by a flow of argon. In the annular plasma, the aerosol is vaporized, atomized, and then ionized. The ionized gasses plus neutral species formed in the annular plasma space are aspirated from the plasma through an orifice into a quadrupole mass spectrometer. The mass range from 1 to 263 amu is rapidly scanned multiple times and ion counts tabulated for mass 59 (cobalt) and mass 69 (gallium). Instrument response is defined by the linear relationship of analyte concentration versus ion count ratio (analyte ion count/ internal standard ion count). Analyte concentrations are derived by reading the ion count ratio for each mass of interest and determining the concentration from the response line. (Houk R, Fassel VA, Flesch GD, et al: Inductively Coupled Argon Plasma as an Ion Source for Mass Spectrometric Determination of Trace Elements. Anal Chem 1980;53:2283-2289)

Specimen Required:
1. Draw blood in a royal blue-top Monoject® trace element blood collection tube(s) - product #8881-307006 (Supply T184).
2. Allow the specimen to clot for 30 minutes; then centrifuge the specimen to separate serum from the cellular fraction.
3. Remove the stopper and carefully pour 2.0 or 1.0 mL of serum into a 7.0-mL, Mayo metal-free, screw-capped, polypropylene vial (Supply T173), avoiding transfer of the cellular components of blood. DO NOT insert a pipet into the serum to accomplish transfer, and DO NOT ream the specimen with a wooden stick to assist with serum transfer.
4. Send specimen refrigerated. All specimens to be stored more than 48 hours should be sent frozen.
5. It is important that the specimen be obtained, processed, and transported according to instructions in "Metals Analysis - Collection and Transport" in Special Instructions in "2002 Test Catalog."

NOTE: Gadolinium imaging media (such as in the contrast agent Omniscan) should not be administered within 48 hours of specimen collection.
Heavy Metals Screen Occupational Exposure, Urine

#82385

Profile Information

<table>
<thead>
<tr>
<th>Unit Code</th>
<th>Reporting Title</th>
<th>Available separately</th>
</tr>
</thead>
<tbody>
<tr>
<td>7243</td>
<td>Creatinine Conc</td>
<td>No</td>
</tr>
<tr>
<td>21669</td>
<td>As Conc (µg/L)</td>
<td>No</td>
</tr>
<tr>
<td>21672</td>
<td>Pb Conc (µg/L)</td>
<td>No</td>
</tr>
<tr>
<td>7244</td>
<td>Cd Conc (µg/L)</td>
<td>Yes</td>
</tr>
<tr>
<td>21666</td>
<td>Hg Conc (µg/L)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Clinical

Arsenic, lead, cadmium, and mercury are well-known toxins and toxic exposures are characterized by increased urinary excretion of these metals.

• Arsenic (As) exists in a number of different forms; some are toxic while others are not. Toxic forms, which are typically encountered as a result of an industrial exposure, are the inorganic species As⁺³ (As-III) and As⁺⁵ (As-V) and the partially detoxified metabolites, monomethylarsine (MMA) and dimethylarsine (DMA). The 2 most common nontoxic forms are arsenobetaine and arsenocholine. Arsenic toxicity affects a number of organ systems.

• Lead (Pb) toxicity primarily affects the gastrointestinal, neurologic, and hematopoietic systems.

• Chronic exposure to cadmium (Cd) causes accumulated renal damage.

• Mercury (Hg) is essentially nontoxic in its elemental form. However, once it is chemically modified to the ionized, inorganic species, Hg⁺², it becomes toxic. Further bioconversion to an alkyl mercury, such as methyl mercury (CH₃Hg⁺), yields a species of mercury that is highly selective for lipid-rich tissue, such as the myelin sheath, and is very toxic.

Useful For

Screening potentially exposed workers for heavy metal toxicity in settings where a 24-hour collection is problematic

Interpretation

• The reference intervals for this test are OSHA thresholds.

• The ordering physician will be contacted regarding any result exceeding OSHA thresholds to determine the level of workplace exposure and follow-up action.

• Arsenic results exceeding the OSHA threshold will be fractionated to confirm the presence of toxic forms.

• Measurement of urine excretion rates either before or after chelation therapy has been used as an indicator of lead exposure. However, blood lead analysis has the strongest correlation with toxicity.

• Normally, the excretion of cadmium is proportional to creatinine. When renal damage occurs, cadmium excretion increases relative to creatinine.

• The correlation between the levels of mercury in the urine and clinical symptoms is poor, but urinary mercury is the most reliable way to assess exposure to inorganic mercury.

Cautions

• Nitric acid cannot be added to either the collection or aliquot container. Nitrate interferes with the extraction procedure that would need to take place in the event of a positive arsenic result.

• This test is intended for use as a screening tool for occupational exposures. It is not a replacement of the 24-hour urine test, #8633 Heavy Metals Screen, Urine.
Test Title: Heavy Metals Screen Occupational Exposure, Urine
#82385

References

Method
All 4 analytes (As, Pb, Cd, and Hg) and analytes individually ordered can be readily analyzed on an inductively coupled plasma mass spectrometer (ICP-MS). Aqueous acidic calibrating standards are diluted with a matrix urine containing normal concentrations of these elements and aqueous acidic diluent containing 3 internal standards. Blanks are diluted with the aqueous acidic diluent containing internal standards, but no urine matrix. Quality control specimens and patient specimens are also diluted in an identical manner. In turn, all diluted blanks, calibrating standards, quality control specimens, and patient specimens are aspirated into a pneumatic nebulizer and the resulting aerosol directed to the hot plasma discharge by a flow of argon. In the annular plasma the aerosol is vaporized, atomized, and then ionized. The ionized gasses plus neutral species formed in the annular plasma space are aspirated from the plasma through an orifice into a quadrupole mass spectrometer. The mass range from 1 to 263 amu is rapidly scanned multiple times and ion counts tabulated for each mass of interest. Instrumentation response is defined by the linear relationship of analyte concentration versus ion count (analyte ion count/internal standard ion count). Analyte concentrations are derived by reading the ion count ratio for each mass of interest and determining the concentration. (Nixon DE, Moyer TP: Routine clinical determination of lead, arsenic, cadmium, and thallium in urine and whole blood by inductively coupled plasma-mass spectrometry. Spectrochimica Acta, Part B-Atomic Spectroscopy 51:13-25. Available from URL: http://toxnet.nlm.nih.gov

Specimen Required:
10 mL from a random urine collection as follows:
1. Collect in a clean, plastic urine collection container.
2. Shake the specimen gently, and pour 10 mL into a plastic, 13-mL urine tube or a clean, plastic aliquot container with no metal cap or glued insert.
3. See “Metals Analysis-Collection and Transport” in Special Instructions for complete instructions.
4. Send specimen refrigerated in a plastic, 13-mL urine tube.

NOTE: Abstain from seafood (especially shellfish) for 2 days prior to urine collection.

Reference Values:
ARSENIC
<50 µg/g creatinine
MERCURY
<35 µg/g creatinine
CADMIUM
<3.0 µg/g creatinine
LEAD
<150 µg/g creatinine

Analytic Time: 2 days
Days Set Up: Monday through Saturday
Fee: $196.30
CPT Code: 82175/Arsenic
82300/Cadmium
83655/Lead
83825/Mercury
Fluconazole (Diflucan) is a synthetic triazole antifungal agent for either intravenous or oral administration. It is indicated for treatment of fungal infections caused by *Candida albicans* and meningitis caused by *Cryptococcus neoformans*.

Fluconazole is <10% protein bound and has a volume of distribution (averaging 0.9 L/kg) indicating that it distributes into tissues.

Fluconazole is cleared predominantly by the kidney. Ninety plus per cent of administered fluconazole is excreted in the urine as parent drug. The apparent elimination half-life is approximately 30 hours (range 20-50 hours). Compromised renal function significantly decreases the rate of elimination and causes fluconazole to accumulate. Distribution and elimination are similar in adults, children as young as age 2 months, and in geriatric patients.\(^1\)\(^2\)

Fluconazole does not undergo extensive hepatic metabolism. However, fluconazole is a potent inhibitor of cytochrome P-450 2C9, which affects drugs metabolized by this system such as benzodiazepines, cyclosporine, hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors, phenytoin, rapamycin, tacrolimus, and warfarin.

Fluconazole is generally well tolerated, but toxicity can occur at high serum concentration. Hepatic toxicity has been observed in renally impaired patients treated with fluconazole. Anaphylaxis, seizures, toxic epidermal necrosis, vomiting, and diarrhea also may occur.\(^3\)

### Useful For
- Monitoring fluconazole therapy to guide dose adjustment and ensure adequate coverage while avoiding high concentrations associated with toxicity, especially in patients who have severely compromised renal function, for those who are undergoing dialysis, or for those where absorption may be impacted.
- Routine drug monitoring is not indicated in most patients.

### Interpretation
Serum concentration of fluconazole correlates with dose (typically 0.1-0.6 gm administered every 24 hours).

- A 0.2-gm dose produces a peak serum concentration (within 30-60 minutes of infusion) of 4.0 to 8.0 µg/mL in adults.
- A 0.4-gm dose produces a peak serum concentration of 8.0-16 µg/mL.

Optimal peak serum concentrations vary according to indication. Peak serum concentrations <4.0 µg/mL are likely to be ineffective, while peak concentrations >20 µg/mL are unlikely to provide increased efficacy and may predispose the patient to toxicity.

### Cautions
Naltrexone and naproxen interfere with fluconazole, causing an artifactual increase in the fluconazole concentration. Pentoxifylline and hydrocodone interfere with the internal standard peak, causing an artifactual decrease in the fluconazole concentration. Physiologic serum concentrations of naltrexone and hydrocodone are not likely to interfere. Naproxen and pentoxifylline are significant interferences.
Test Title: Fluconazole, Serum
#82522

References

Method

Specimen Required: Draw blood in a red-top tube(s). Spin down and send 2.0 mL serum refrigerated.
DATE, TIME OF PATIENT'S LAST DOSE, AND DOSE AMOUNT ARE REQUIRED ON REQUEST FORM FOR PROCESSING.

Reference Values: 4.0-20 µg/mL.
Analytic Time: Same day / 1 day
Days Set Up: Monday through Friday
Fee: $114.70
CPT Code: 80299
Mercury for Occupational Monitoring, Urine
#82755

Profile Information

<table>
<thead>
<tr>
<th>Unit Code</th>
<th>Reporting Title</th>
<th>Available separately</th>
</tr>
</thead>
<tbody>
<tr>
<td>7243</td>
<td>Creatinine Concentration</td>
<td>No</td>
</tr>
<tr>
<td>21666</td>
<td>Hg Conc (µg/L)</td>
<td>No</td>
</tr>
</tbody>
</table>

Clinical

Mercury (Hg), a well-known toxin, is essentially nontoxic in its elemental form. However, once it is chemically modified to the ionized, inorganic species, Hg⁺, it becomes toxic. Further bioconversion to an alkyl mercury, such as methyl mercury (CH₃Hg⁺), yields a species of mercury that is highly selective for lipid-rich tissue, such as the myelin sheath, and is very toxic.

Industrial exposure is a major source of mercury intoxication.

Useful For

Screening potentially exposed workers for mercury toxicity in settings where a 24-hour collection is problematic

Interpretation

- Urinary mercury is the most reliable way to assess exposure to inorganic mercury, but the correlation between the levels of excretion in the urine and clinical symptoms is poor.
- The reference interval corresponds to the OSHA guideline for mercury exposure.
- The ordering physician should be contacted regarding any result exceeding OSHA thresholds to determine the level of workplace exposure and follow-up action.

Cautions

Nitric acid should not be added to the collection. This test is intended for use as a screening tool for occupational monitoring. It is not a replacement of the 24-hour urine test, #8633 Heavy Metals Screen, Urine.

References

Method

Mercury can be readily analyzed on an inductively coupled plasma mass spectrometer (ICP-MS). Aqueous acidic calibrating standards are diluted with a matrix urine containing normal concentrations of these elements and aqueous acidic diluent containing 3 internal standards. Blanks are diluted with the aqueous acidic diluent containing internal standards but no urine matrix. Quality control specimens and patient specimens also are diluted in an identical manner. In turn, all diluted blanks, calibrating standards, quality control specimens, and patient specimens are aspirated into a pneumatic nebulizer and the resulting aerosol directed to the hot plasma discharge by a flow of argon. In the annular plasma the aerosol is vaporized, atomized, and then ionized. The ionized gasses plus neutral species formed in the annular plasma space are aspirated from the plasma through an orifice into a quadrupole mass spectrometer. The mass range from 1 to 263 amu is rapidly scanned multiple times and ion counts tabulated for each mass of interest. Instrumentation response is defined by the linear relationship of analyte concentration versus ion count (analyte ion count/internal standard ion count). Analyte concentrations are derived by reading the ion count ratio for each mass of interest and determining the concentration. (Nixon DE, Moyer TP: Routine clinical determination of lead, arsenic, cadmium, and thallium in urine and whole blood by inductively coupled plasma mass spectrometry. Spectrochimica Acta, Part B-Atomic Spectroscopy 51:13-25. Available from URL: [http://toxnet.nlm.nih.gov](http://toxnet.nlm.nih.gov))

Specimen Required:
10 mL from a random urine collection as follows:
1. Collect in a clean, plastic urine collection container.
2. Shake the specimen gently, and pour 10 mL into a plastic, 13-mL urine tube or a clean, plastic aliquot container with no metal cap or glued insert.
3. See “Metals Analysis-Collection and Transport” in Special Instructions for complete instructions.
4. Send specimen refrigerated in a plastic, 13-mL urine tube.

NOTE: Abstain from seafood (especially shellfish) for 2 days prior to urine collection.

Reference Values:
Mercury/Creatinine Ratio:
<35 µg/g creatinine

Analytic Time: 2 days
Days Set Up: Monday through Saturday
Fee: $98.10
CPT Code: 83825
Hepatitis C Virus (HCV) Quantitation by Reverse Transcription-Polymerase Chain Reaction (RT-PCR), Serum #83142

Clinical

Hepatitis C virus (HCV) is recognized as the cause of most cases of posttransfusion hepatitis and is a significant cause of morbidity and mortality worldwide. In the United States, HCV infection is quite common, with an estimated 3.5-4 million chronic HCV carriers.

In patients with chronic HCV, the response to interferon-alpha therapy is correlated with pretreatment serum or plasma HCV RNA levels. Similarly, the optimal duration of combination interferon-ribavirin therapy can be determined from the patient's pretreatment viral load (and the HCV genotype). Preliminary studies have suggested that changes in HCV RNA viral load after the initiation of therapy may be useful for predicting a sustained response to antiviral therapy.

Useful For

Quantifying HCV levels to help predict treatment response and to determine treatment duration

Note: Mayo Medical Laboratories (MML) has identified #81130 Hepatitis C Virus (HCV) Quantitation by bDNA, Serum as the preferred test for quantifying HCV levels (see “CAUTIONS”)

Interpretation

A positive result is expressed as a quantitative value in HCV IU/mL and indicates the presence of quantifiable HCV RNA. A negative result (<600 IU/mL) indicates that either the specimen contains no HCV RNA or that the levels of HCV RNA are below the detection limits of the assay. Current information in the literature indicates that 800,000 IU/mL of HCV RNA is the proposed threshold value for tailoring the duration of combination therapy.

Cautions

• This assay has been shown to have a limited upper linear range. In order to obtain a quantitative value from patients who have not received treatment (and may therefore have high levels of viral RNA present), this assay requires specimen dilution and repeat testing in a majority of the specimens. Discrepancies have also been identified in the upper range supporting the suggestion that the assay is operating beyond its linear range using the cutoff of 500,000 IU/mL suggested by the manufacturer.

• MML has identified the #81130 Hepatitis C Virus (HCV) Quantitation by bDNA, Serum (Bayer VERSANT HCV v3.0) as our preferred quantitative test because of its wider dynamic range and comparable sensitivity.

• For serial monitoring of HCV viral loads, use of a consistent testing methodology is recommended.

• The use of this test should be restricted to HCV seropositive patients who tested positive in at least 1 previous qualitative RT-PCR or equivalent assay. This assay should never be used as a primary screening test. A negative result does not always indicate the absence of HCV (see “INTERPRETATION”).

• Improper specimen collection, handling, or storage may invalidate test results.

Supportive Data

A comparison of quantitative results expressed in HCV IU/mL, obtained from the VERSANT HCV RNA 3.0 (bDNA-3.0) assay, the QUANTIPLEX HCV RNA 2.0 (bDNA-2.0) assay, and the COBAS AMPLICOR HCV MONITOR version 2.0 (HCM-2.0) test was performed. A total of 168 patient specimens submitted to the Mayo Clinic Molecular Microbiology Laboratory for HCV quantification or HCV genotyping was studied. Of the specimens tested, 97%, 88%, and 79% yielded quantitative results within the dynamic range of the bDNA-3.0, bDNA-2.0, and HCM-2.0 assays, respectively. Overall, there was a substantial agreement between the results generated by all 3 assays. A total of 15 out of 29 (52%) of the specimens determined to contain viral loads of <31,746 IU/mL by the bDNA-3.0 assay were categorized as containing viral loads within the range of 31,746 to 500,000 IU/mL by the bDNA-2.0 assay. Although substantial agreement was noted between the results generated by the bDNA-2.0 and bDNA-3.0 assays, a bias toward higher viral titer by the bDNA-2.0 assay was noted ($P=0.001$).
Method

The Roche COBAS Amplicor HCV Monitor v2.0 test is an automated system and is based on 4 major processes: specimen preparation; RT-PCR amplification of target RNA using HCV specific complementary primers; hybridization of the amplified DNA (PCR product) to oligonucleotide probes specific for the target(s); and detection of the probe-bound amplified products by colorimetric determination.

Roche COBAS Amplicor HCV Monitor v2.0 test permits simultaneous RT-PCR amplification of HCV target and HCV Quantitation Standard (QS) target DNA. The quantitation of HCV viral RNA is performed using the HCV QS. The HCV QS is noninfectious plasmid DNA that contains the identical primer binding sites as the HCV RNA target and a unique probe binding region that allows QS amplicon to be distinguished from HCV amplicon. The QS is incorporated into each individual specimen at a known copy number and is carried through the specimen preparation, RT-PCR amplification, hybridization, and detection steps along with the HCV target and is amplified together with the HCV target. The Master Mix reagent contains a primer pair of specific for both HCV and HCV QS sequences. The COBAS AMPLICOR Analyzer calculates the HCV RNA level in the test specimens by comparing the HCV signal to the QS signal for each specimen. (Germer JJ, Heimgartner PJ, Ilstrup DM, et al: Comparative Evaluation of the Bayer HCV RNA 3.0, Bayer HCV RNA 2.0, and COBAS AMPLICOR HCV MONITOR Version 2.0 Assay for the Quantification of Hepatitis C Virus RNA is Serum. J Clin Microbiol 2002;40:495-500)

Specimen Required:

Draw blood in an SST tube(s). Aseptically spin down within 4 hours of draw, and send 1.0 mL of serum frozen in a screw-capped, sterile, plastic vial. Maintain sterility and forward promptly. See “Infectious Material” in Special Instructions.

NOTE: 1. THIS TEST IS INTENDED TO BE USED TO MONITOR KNOWN HCV POSITIVE INFECTIONS. IT IS NOT INTENDED FOR PRIMARY DETECTION OF HCV INFECTIONS.
2. THE FOLLOWING SPECIMENS WILL NOT BE TESTED:
   A. FREEZE/THAW CYCLES
   B. OBVIOUS MICROBIAL CONTAMINATION
   C. PROLONGED AMBIENT OR REFRIGERATE TEMPERATURE EXPOSURE.
   D. SPECIMEN-TO-SPECIMEN CONTAMINATION
   E. RED TOP SERUM
   F. ACD PLASMA
3. Please complete a “Microbiology Request Form” (Supply T244) or a “MayoConnect Additional Test Information Form” (Supply T357) supplied and forward it with the specimen.

Reference Values:
If detected, results are reported in IU/mL and Base 10 Logarithm.

The lower limit of detection of HCV RNA by this method is 600 IU/mL. A result of <600 IU/mL does not rule out the presence of HCV RNA.

Analytic Time: Same day/1 day
Days Set Up: Monday through Friday
Fee: $349.00
CPT Code: 87522