Access
Immunoassay Systems

HAV IgM
REF 34210

Intended Use

The Access HAV IgM assay is a paramagnetic-particle, chemiluminescent immunoassay for the detection of IgM antibody to hepatitis A virus in human serum and plasma using the Access Immunoassay Systems.

Summary and Explanation

The hepatitis A virus, described for the first time in 1973 by Feinstone, is an enteric RNA virus, with a 27 nm diameter, belonging to the Picornaviridae group.[1,2] The virus transmission is fecal-oral. It is excreted in the feces, mainly at the very beginning of the disease.[3] The pathology induced by the virus is characterized by a clinical picture from inapparent infection to clinical icteric or anicteric hepatitis and appears sometimes under the form of a fulminant hepatitis, the age being one of the main factors of the disease severity.

Epidemiological studies have shown that several endemy areas exist. It turns out that endemy is mainly function of hygiene and sanitary conditions met in different geographic areas. In industrialized countries, a seroprevalence decrease has been noted since a few years; on the other hand, it is accompanied by an increase of severe clinical forms. The recent vaccine appearance allowed to provide a long term protection for groups at risk. Currently in Europe, travelers going to high endemic areas and community employees (food industry, health staff) are vaccinated. The Access HAV IgM assay allows the detection of IgM antibody and is applied to the serological diagnosis of acute hepatitis. This diagnosis is based on the detection of anti-HAV IgM antibody which appear from the first symptoms of the disease and persist during the three to six months of convalescence.[4,5,6]

Principles of the Procedure

The Access HAV IgM assay is an immunoassay based on the principle of immunocapture. The sample to be tested is added to a reaction vessel with paramagnetic particles coated with anti-human IgM antibody (sheep polyclonal) as capture antibody. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then a complex formed with hepatitis A virus antigen and specific hepatitis A monoclonal antibody,[7] labeled with alkaline phosphatase, is added to the reaction vessel. After a second incubation and a second washing, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the enzymatic reaction is measured with a luminometer. The light production is a function of the amount of enzyme conjugate present at the end of the reaction. The light quantity measured for a sample allows to determine the presence or the absence of anti-HAV IgM antibody, by comparison with a cut-off value defined during the assay calibration on the instrument. If the light production is equal to or greater than the cut-off value, the sample is considered positive for the Access HAV IgM assay.

Product Information

Access HAV IgM Reagent Packs

Cat. No. 34210: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C .
- Stable at 2 to 10°C for 28 days after initial use.

- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.

R1a:	Paramagnetic particles coated with anti-human IgM antibody (sheep polyclonal) suspended in TRIS buffered saline, with surfactant, BSA, < 0.1% sodium azide and 0.1% ProClin** 300.
R1b:	Conjugate: hepatitis A Ag (inactivated) - Mouse anti-hepatitis A virus monoclonal Ab - alkaline phosphatase (bovine) complex in TRIS with surfactant, BSA, < 0.1% sodium azide. **Note:** The hepatitis A virus antigen is inactivated by chemical treatment (formaldehyde).

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.[8]
- Xi. Irritant: 0.1% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. Serum and plasma (heparin, EDTA or citrate) are the recommended samples. The blood preservatives ACD, CPD and CPDA are compatible with the assay.
2. Observe the following recommendations for handling, processing, and storing blood samples:[9]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter have been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

5. Samples containing up to 80 mg/L bilirubin or up to 200 g/L albumin, lipemic samples containing the equivalent of 36 g/L triolein and hemolyzed samples containing up to 10 g/L hemoglobin do not affect the result.

Materials Provided

R1 Access HAV IgM Reagent Packs

Materials Required But Not Provided

1. Access HAV IgM Calibrators
   Negative and positive for anti-HAV IgM antibody.
   Cat. No. 34215
2. Quality control (QC) materials: Access HAV IgM QC or other commercially available quality control material
   Cat. No. 34219
3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use ten (10) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The first result is obtained in 35 minutes.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Calibration Details

Calibration data determining the assay cut-off value is valid for 28 days. Refer to the appropriate system manuals and/or Help system for complete instructions on calibration procedures.

Quality Control

Quality controls are recommended at least every 24 hours and on the system start-up prior to running patient samples.[10] More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Use the suggested product Access HAV IgM QC, or include quality control sera from other sources. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results

Patient test results are determined automatically by the system software using the cut-off value determined by the active calibration. Results are reported reactive or non-reactive in function of their ratio with the cut-off value (signal greater than or equal to the cut-off value; the cut-off

value is 1.0). Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results. In the scope of an acute hepatitis diagnosis, it is recommended to retest patients with low reactive results (result 1-2 times greater than the cut-off value) after 1 week in order to make the distinction between an acute infection (IgM level increased) and an hepatitis in convalescence phase (stable or decreased IgM level).

Limitations of the Procedure

1. The Access HAV IgM assay is strictly limited to the detection of anti-HAV IgM antibody in human serum and plasma.
2. The serological diagnosis of acute hepatitis A can not be only based on the IgM antibody detection using the Access HAV IgM assay. Other biological markers as well as clinical symptoms and patient history are necessary to establish such a diagnosis.
3. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[11,12]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
4. The Access HAV IgM results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.

Specific Performance Characteristics

Results below mentioned were generated in one internal and one external site.

Intra-assay imprecision

Intra-assay imprecision was determined with a panel of 4 sera defined as follows:

S1 S/CO ratio 0.05
S2 S/CO ratio 8.5
S3 S/CO ratio 32
S4 S/CO ratio 43

Each serum was tested 30 times in the same run. All the intra-assay variation coefficients are lower than or equal to 5% (Table 1).

Table 1: Intra-assay imprecision of the Access HAV IgM assay

Panel	Mean (n=30) S/CO ratio	CV%
Serum 1	0.05	2.1
Serum 2	8.49	5.0
Serum 3	32.11	4.8
Serum 4	43.24	4.3

Inter-assay imprecision

Inter-assay imprecision (5 replicates, twice a day, 5 different days) performed on the same sera provided variation coefficient values ranging from 4.5% to 13.4%, (Table 2).

Table 2: Inter-assay imprecision of the Access HAV IgM assay

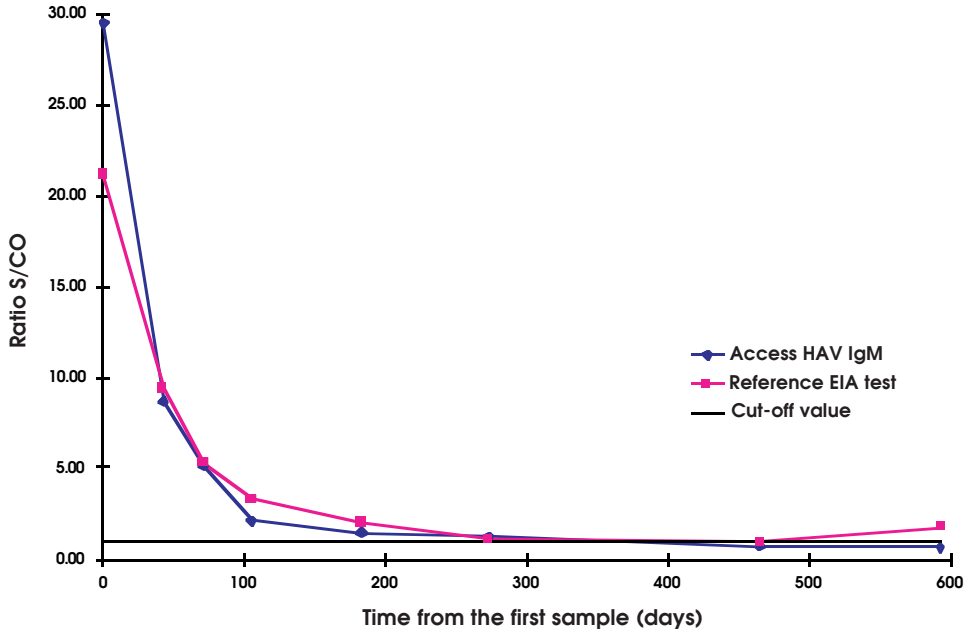
Panel	Mean (n=30) S/CO ratio	CV%
Serum 1	0.06	13.4
Serum 2	8.00	6.8
Serum 3	32.42	4.5
Serum 4	43.37	8.1

Sensitivity

Access HAV IgM assay sensitivity was studied on a population of 210 individual samples and on 157 samples from 26 patients with acute hepatitis A. Results obtained with the Access HAV IgM assay were compared with a commercially available EIA technique and discrepant results were confirmed using a third EIA method.

The study of the 26 seroconversion or clinical follow-up cases shows 100% sensitivity on the first positive serum of each patient. Results obtained on individual sera show 99% sensitivity (208/210) versus the EIA kit used in reference and after confirmation with the third EIA kit.

Figure 1: Blood samples from one patient with hepatitis A



Specificity

Specificity was studied on a population of 706 blood donors, 368 in-patients and on a population of 461 young soldiers. Results obtained were compared with results from a commercialized EIA technique and discrepant results were confirmed using a third EIA method.

Out of the total of the 1535 tested samples, only one was found repeated positive resulting in 99.93% specificity.

Specificity – Selected Populations

Specificity of the Access HAV IgM assay was also evaluated on samples positive for different viral and parasitic infections, on samples positive for rheumatoid factor and autoantibodies, and on samples from pregnant women and haemodialysed patients.

Specificity was 99.53% (427/429).

Table 3: Specificity of the Access HAV IgM assay (selected population)

Pathology	Number of samples tested	Reactive samples	Specificity %
Autoimmune dieases (ANA)	**10**	**0**	
Viral and parasitic infections including:			
Chagas	2	0	
anti-HAV IgG	10	0	
anti-HBs	10	0	
HBc Ab + (HBs Ag -)	10	0	
HBc IgM + (HBs Ag +)	9	0	
anti-HCV	10	0	
anti-*T. gondii* (IgM)	10	0	
anti-*T. gondii* (IgG)	10	0	
anti-Rubella (IgM)	10	0	
anti-Rubella (IgG)	10	0	
anti-Mumps (IgM)	10	0	
anti-Mumps (IgG)	10	0	
anti-Measles (IgM)	10	2	
anti-Measles (IgG)	10	0	
anti-CMV (IgM)	10	0	
anti-CMV (IgG)	10	0	
anti-EBV (IgM/VCA)	10	0	
anti-EBV (IgG)	10	0	
anti-HSV (IgM)	7	0	
anti-HSV (IgG)	15	0	
anti-VZV (IgM)	10	0	
anti-VZV (IgG)	10	0	
anti-HIV 1/2	10	0	
Yellow fever virus	10	0	
Poliovirus	35	0	
Heterophil Ab (HAMA)	11	0	
Other origins:		0	
Pregnant women	21	0	
Rheumatoid Factor	63	0	
Myeloma	10	0	
Haemodialysed Patients	46	0	
TOTAL	**429**	**2**	**99.53%**

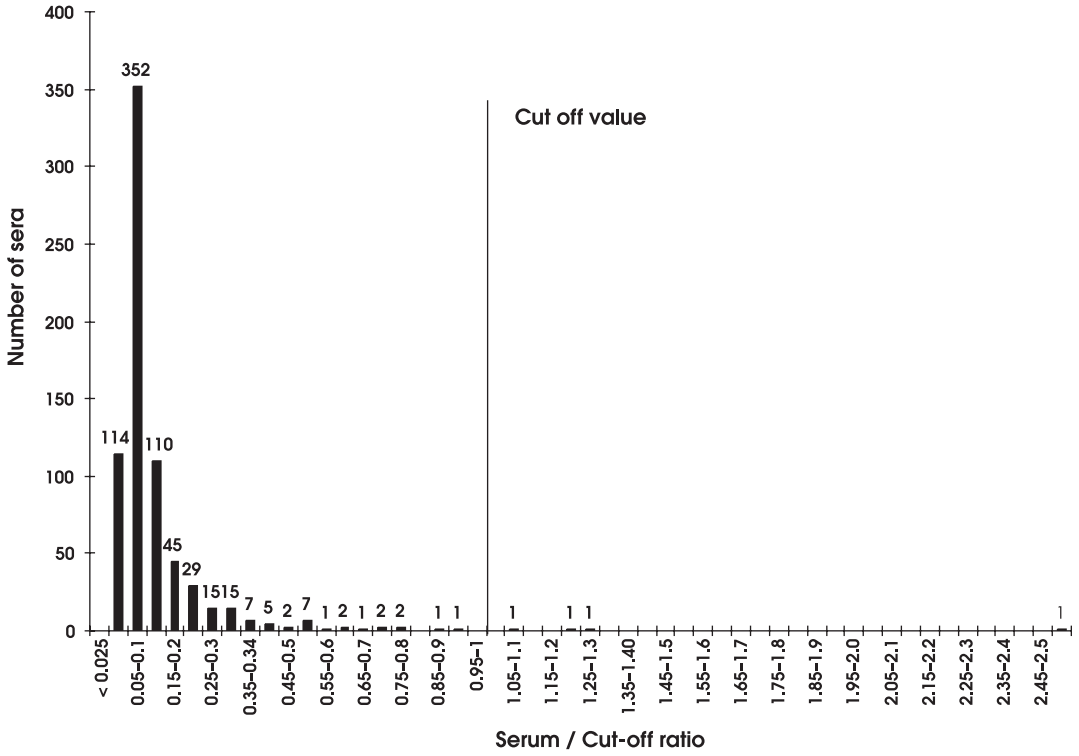
Interference

A study of anticoagulant interference was made in our laboratories; 85 plasmas were studied without any significant difference demonstrated between the raw signals obtained on plasma and on serum, (65 on EDTA, 20 in parallel on ACD, CPDA, CPD, citrate and heparin).

Prevalence

The prevalence study made on 715 blood donors (Paris area, France) is 0.56% (4 positives/715).

Figure 2: Study of prevalence on a blood donor population, N=715



Access
Immunoassay Systems

HAV IgM CALIBRATORS

REF 34215

Intended Use

The Access HAV IgM Calibrators are intended for use with the Access HAV IgM assay for the detection of IgM antibody to hepatitis A virus antigen in human serum and plasma using the Access Immunoassay Systems.

Summary and Explanation

The Access HAV IgM Calibrators are used to establish calibration (determine the cut-off value) for the Access HAV IgM assay. By comparing the light intensity generated by a sample to the cut-off value, it is possible to determine the presence or absence of anti-HAV IgM antibody in the sample.

Traceability

The measurand (analyte) in the Access HAV IgM Calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access HAV IgM Calibrators

Cat. No. 34215: C0 and C1, 1.0 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Signs of possible deterioration are quality control values out of range.

C0:	Negative calibrator: human defibrinated plasma negative for anti-HAV IgM antibody, < 0.1% sodium azide.
C1:	Positive calibrator: human defibrinated plasma positive for anti-HAV IgM antibody, < 0.1% sodium azide.
Calibration Card:	1

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[13]

- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[8]
- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibration Details

The Access HAV IgM Calibrators are provided as:

C0 negative (non-reactive) for anti-HAV IgM antibody
C1 positive (reactive) for anti-HAV IgM antibody

The Access HAV IgM assay requires a calibration (determination of the cut-off value) every 28 days in order to have an active calibration. A calibration of the Access HAV IgM assay requires 200 µL (5 drops/cup) of each calibrator (cut-off value determined by running C0 in duplicate and C1 in triplicate).

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

Access
Immunoassay Systems

HAV IgM QC

REF 34219

Intended Use

The Access HAV IgM QC is intended for monitoring system performance of the Access HAV IgM assay.

Summary and Explanation

The Access HAV IgM QC is intended for the use in clinical laboratories as quality control materials to monitor system performance of the Access HAV IgM assay for anti-HAV IgM antibody detection. The use of quality control materials is indicated for detecting and potentially resolving critical testing errors due to test kits, personnel and instrumentation and are an integral part of good laboratory practices.[10,14,15,16,17,18] A negative quality control and a positive quality control are provided to allow performance monitoring in the most relevant areas of the assay range.

Traceability

The measurand (analyte) in the Access HAV IgM QC is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of QC and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access HAV IgM QC

Cat. No. 34219: 2.5 mL/vial, 3 vials each level

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Vial is stable at 2 to 10°C for 30 days after initial use.
- Signs of possible deterioration are quality control values out of range.

QC 1:	Human defibrinated plasma, negative (non-reactive) for anti-HAV IgM, < 0.1% sodium azide.
QC 2:	Human defibrinated plasma, positive (reactive) for anti-HAV IgM, < 0.1% sodium azide.
QC Value Card:	1

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[13]
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[8]
- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure

The Access HAV IgM QC should be treated in the same manner as patient specimen and run in accordance with the instructions accompanying the instrument being used. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[10] More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

Note: For the Access Immunoassay Systems, refer to the appropriate system manuals and/or Help system for information on quality control theory, configuring quality controls, quality control sample test request entry, and reviewing quality control data.

To process a single determination of Access HAV IgM QC on the Access Immunoassay Systems, a minimum of 160 µL (4 drops/cup) is required for each control.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

Expected Values

The expected control values for the Access HAV IgM assay are provided on the QC Value Card contained in the Access HAV IgM QC assay kit. Results obtained by the user laboratories should fall within the stated ranges. Variations, such as in technique, equipment, or reagents may result in values different from those listed. However, each laboratory should establish its own mean value and acceptable ranges after sufficient data has been collected.[18]

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Beckman Coulter, Inc.
250 S. Kraemer Blvd.
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