The buffer is a tris-barbital-sodium barbital buffer. The packaged buffer should be stored at
Serum is the specimen of choice. Plasma collected in
The buffer contains barbituric which, in sufficient quantity, can be toxic. Preparation for Use: Dissolve one package in 750 mL deionized water. The buffer is ready for use when all material is completely dissolved.

Storage and Stability: The package buffer should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. Diluted buffer is stable for two months at 15 to 30°C. Signs of Deterioration: Discard packaged buffer if the material appears cloudy or discoloration or sediment. Discard diluted buffer if it becomes turbid.

4. Titan® III Plates (Cat. No. 3001) Ingredients: Cellulose acetate WARNING: FOR IN-VITRO DIAGNOSTIC USE Preparation for Use: The plates are ready for use as packaged. Storage: The plates should be stored at 15 to 30°C.

INSTRUMENTS Any in-vitro scanning densitometer with visible capabilities may be used.

SPECIMEN COLLECTION AND HANDLING Specimen: Serum is the specimen of choice. Plasma collected in heparin may be used. Anticoagulants containing oxalate, citrate or EDTA cannot be used because these substances inhibit the alkaline phosphatase activity.

Patient Preparation: The patient should be fasting. Patients who have B or O blood group and are secretors may have an elevated alkaline phosphatase about two hours after a fatty meal.

Interfering Substances: 1. High concentrations of phosphate, oxalate, citrate and cyanide will inhibit ALP activity. 2. Excess glycine may inhibit ALP activity by complexing magnesium. 3. EDTA inhibits some of the isoenzymes of ALP. Do not use as an anticoagulant. 4. Several drugs cause an enzymatic imbalance which may change the ALP level.

Storage and Stability: It is preferable to refrigerate the blood specimen immediately after collection. Specimens should be separated from the red blood cells as soon as possible. It is strongly recommended that fresh serum samples be used. If storage is necessary, the serum should be stored frozen (-20°C) for no more than 24 hours.

PROCEDURE Materials Provided: The following materials are necessary for use in the Alkaline Phosphatase Isoenzyme Test:

1. Preparation for Use: Reconstitute each vial of reagent with 3 mL of Diluent. Mix the reagent to obtain complete dissolution. The reagent is to be used as soon as reconstituted or within 48 hours.

2. Storage and Stability: The reagents should be stored at 2 to 8°C and are stable until the expiration date indicated on the label. The reconstituted reagent is stable 48 hours.

3. Signs of Deterioration: The dry, unconstituted reagent should be uniformly off-white to light lavender in color.

2. Alkaline Phosphatase Indoly/ Blue Diluent Ingredients: 2-Amino-2-methyl-1-propanol and Magnesium Chloride

3. Signs of Deterioration: Discard the diluent if it shows signs of bacterial growth.

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Isolated from the sera of patients with neo
Because of the similarities to placental isoenzyme, it has been referred to as carcinoplacental isoenzyme. Regan has been quoted as saying he isolated PLACENTAL ISOENZYME: Appears in the serum of pregnant women and in the sera of the babies of both normal and diabetic pregnancies. It has also been observed in cases of metastatic carcinoma to the pleural surfaces and in adenocarcinoma of the pancreas.

Bone; being extremely heat sensitive, it will be inactivated by heat inactiva

LIVER ISOENZYMES: Normally seen in the serum of patients who have B or O blood types, especially after a fatty meal. Patients may have a latent disease process present in the form of a low-grade inflammatory disease. Ulcerative diseases of the intestine and faintly in liver cirrhosis, as well as some inflammatory bowel diseases.

BONE ISOENZYME:

REGAN ISOENZYME: A rare isoenzyme reported by Nerenberg and Kranz which, like the Regan isoenzyme, migrates to the placental position. This isoenzyme represents a disease state of the kidneys or rejection of kidney transplant.

REGAN ISOENZYME: Isolated from the sera of patients with neoplastic diseases and from a condition that is similar to placental isoenzyme has been referred to as carcinoplacental isoenzyme. Regan has been isolated from patients with lung cancer, breast cancer, ovarian cancer, and carcinoma of the colon.

NAGAO ISOENZYME: A variant of Regan isoenzyme that migrates in the alpha and/or beta regions of the alphas serum. It has been isolated in metastatic carcinoma to the pleural surfaces and in adenocarcinoma of the pancreas or bile duct.

PA ISOENZYMES: A variant of the alphas serum that is observed in sera of patients with pancreatic cancer. Chao observed the band in 16 patients (15 with carcinoma of the pancreas and one with cholangiocarcinoma).

ULTRA-FAST BAND: A band migrating in the albumin position on cel-

FIGURE 1: Schematic representation of the electrophoretic mobility of alkaline phosphatase isoenzymes compared to serum proteins.

Figure 2: Helena Iso-Vi-Vis plate showing the migration patterns of the alkaline phosphatase isoenzymes.

The majority of liver isoenzyme (in the αi position) is the most consistently elevated when total ALP levels are elevated. 11 The αi liver ALP increases in the blood early in liver disease and is therefore considered abnormal. An extensive group of conditions lead to increased αi liver ALP including acute hepatitis, cirrhosis, fatty liver, drug induced liver disease, obstruction of biliary flow by carcinoma at the head of the pancreas, bile duct stricture, primary biliary cirrhosis, and metastatic carcinoma to the liver.

Fast liver (in the αi position) has been isolated in cases of metastatic carcinoma to the pleural surfaces and in adenocarcinoma of the pancreas.

Further Testing and Special Treatment of the Serum Specimen: 1. Total alkaline phosphatase activity should be determined on all samples. 

Neoplasms may cause an increase in liver ALP. The increase may be due to an increase in the amount of liver ALP released into the blood stream, or an increase in the rate of synthesis of liver ALP.

Pathologically, the band may be present in perforation of the bowel, and chemical inhibition: 

An abnormally high bone isoenzyme level may also be indicative of bone cancer, osteomalacia or coeliac sprue. A transient increase in the serum of children may be attributed to osteitis or to hypophosphatasia.

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STEP-BY-STEP METHOD
A. Preparation of Titan® III Plate
1. Properly code the required number of Titan® Plates by marking on the glossy, hard side with a Helena Marker. Place a mark in the corner of each plate.
2. Dissolve one bag of Electra® HR Buffer in 750 mL of distilled water.
3. The plates should be soaked in the Bufferizer for 30 minutes according to the instructions for use. Alternatively, the plates may be welled by slowly and uniformly lowering a rack of plates into the buffer. A filtering funer may be used for soaking up to 12 plates, or for approximately one week if stored tightly closed. Improper storage may cause poor separation of the isoenzymes.
B. Preparation of Zone II® Chamber
1. Pour approximately 100 mL of buffer into each of the outer sections of the chamber.
2. Wet two chamber wicks in the buffer and drape one over each support bridge, being sure to contact the buffer with the wick and that there are no air bubbles under the wick.
3. Cover the chamber to prevent buffer evaporation. Discard the buffer after use.
C. Sample Application
Place 10 µL of sample into each well of the Sample Well Plate using the Microdispenser. Cover the Sample Well Plate with a glass slide if the samples are not used within 2 minutes.
2. Prime the Super Z Applicator by quickly depressing the tips into the sample wells 3 or 4 times. Apply this loading to a piece of filter paper. Do not load the applicator again at this point, but proceed immediately to the next step. Priming the applicator makes the next loading much faster.
3. Remove the wet Titan® III Plate from the buffer with the fingers of your dominant hand and quickly place it on the center of the aligning base to prevent the plate from shifting during the superimposing step. Place the plate in the Aligning Base, cellulose acetate side up.
4. Apply the sample to the plate by depressing the applicator tips into the sample wells 3 or 4 times and promptly transferring the applicator to the Aligning Base. Press the button down and hold it for 5 seconds. Make 2 to 3 superimposed applications by repeating this step.
D. Electrophoresis of Sample Plate
1. Quickly place the plate on the superimposed applications in the Aligning Base. Press the button down and hold it for 5 seconds. Make 2 to 3 superimposed applications by repeating this step.
2. Electrophoresis for 20 minutes at 180 volts.
E. Visualization of Isoenzymes Bands
1. Electrophoresis may be complete by placing the chamber at the end of the electrophoresis period and blotting lightly. Place the plate, cellulose acetate side up, on the blotter. Pipette 5 mL of 1% reagent onto the blotter. Place the plate on the blotter with the cellulose acetate side toward the surface of the blotter. Allow the reagent to soak into the plate for 1 minute.
2. Lay a clean glass rod or a serological pipette on the cellulose acetate, and gently roll it across the plate to remove any excess reagent. Failure to remove sufficient excess reagent or excessive pressure on the rod will cause smearing of the pattern.
3. Place the plate, acetate side up, into a preheated Incubation Chamber for 30 minutes at 37°C.
4. After incubation, place the plate in a staining rack, and immerse in 5% acetic acid for 5 minutes.
5. Then, immerse the plate and rack in water for 5 minutes.
6. Remove the plate from the rack and lay it on a blotter. Dry it in a stream of room temperature air for 10 minutes.
F. Evaluation of the ALP bands
1. Qualitative evaluation: The ALP plates may be inspected visually for the presence of the isoenzyme bands.
2. Quantitative evaluation: Scan the dried visible ALP plates by placing them on the center of the aligning base to prevent the plate from shifting during the superimposing step. Priming the applicator makes the next loading much faster.

SUMMARY OF CONDITIONS

<table>
<thead>
<tr>
<th>Incubation Temperature</th>
<th>Voltage</th>
</tr>
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<tbody>
<tr>
<td>30°C</td>
<td>180 V</td>
</tr>
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</table>

Figure 1: Schematic representation showing the migration patterns of the alkaline phosphatase isozyme compared to serum proteins.

Figure 2: Helena Iso-Vi-Vis plate showing the migration patterns of the alkaline phosphatase isozymes.

Alkaline Phosphatase Isozyme Control

<table>
<thead>
<tr>
<th>Anode</th>
<th>Cathode</th>
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<tbody>
<tr>
<td>L</td>
<td>L</td>
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<td>B</td>
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<td>L</td>
<td>L</td>
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<tr>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Ultra Fast Band (UF)</td>
<td>Intestine (I)</td>
</tr>
</tbody>
</table>

RESULTS

Results of the alkaline phosphatase isoenzyme migration are described in comparison to typical serum protein migrations. Fast liver (pre-liver) migrates in the protein alpha, region and the major liver bands migrate in the alphal, beta, and pre-beta regions. Placental, Regan, and renal isoenzymes migrate with the alpha, beta, and pre-beta region, but they appear as tighter bands than bone. PA migrates cathodic to the liver.

INTERPRETATION OF RESULTS
LIVER ISOENZYMES: The major liver isoenzyme (in the pi region) is the isoenzyme that becomes most frequently elevated when total ALP levels are elevated. 14 The alpha liver ALP increases in the blood early in liver disease, before other markers for disease, such as alpha fetoprotein. An extensive group of conditions lead to increased alpha liver ALP including acute hepatitis, cirrhosis, fatty liver, drug induced liver disease, obstruction of biliary flow by carcinoma at the head of the pancreas, bile duct stricture, primary biliary cirrhosis, and metastatic carcinoma to the liver.

The major liver ALP is the most commonly elevated in patients with viral hepatitis, alcoholic cirrhosis and other liver diseases. Data generated in a study by Viot and his associates 15 suggests that alpha liver ALP is highly correlated with the presence of liver metastases and that the presence of this isoenzyme could be predictive of the appearance of liver metastases. Viot also reports that fast liver is seen occasionally in patients free of any disease state.16

BONE ISOENZYME: Elevated as result of osteosclerotic disease. May be elevated in growing children and adults over the age of 60. The highest total ALP values have been attributed to the fast liver isoenzyme due to Paget’s disease or renal rickts. 16 An abnormally high bone isoenzyme level may also be indicative of bone cancer, osteomalacia or coeliac sprue. An elevated bone isoenzyme in children may be attributed to crenitis or to hypoplasia.

PLACENTAL ISOENZYME: Appears in the serum of pregnant women late in the first trimester of pregnancy and may be elevated for one month after termination of pregnancy. 17 Infarction of the placenta in a previous pregnancy or a placental plasm. INTESTINAL ISOENZYME: Normally seen in the serum of subjects who have B or B type blood, especially after a fatty meal. Pathologically, the band may be present in the bile ducts or may be of the hepatic or biliary duct systems. The band is useful in detecting the ulcerative diseases of the intestine and fatty liver in cirrhosis, as well as in some instances of the malabsorption syndrome. REAIL ISOENZYME: A rare isoenzyme reported by Nerenberg 18 and Kranck 26 which, like the Regan isoenzyme, migrates to the placental position. This isoenzyme represents a disease state of the kidneys or rejection of kidney transplant.

REGAN ISOENZYME: Isolated from the sera of patients with neo-plastic disease. The presence of the isoenzyme in the serum has been referred to as carcinoplacental isoenzyme. Regan has been isolated in patients with lung cancer, breast cancer, ovarian cancer, and carcinoma of the colon.

NAGAO ISOENZYME: A variant of Regan isoenzyme that migrates in the alpha and beta region. Regan on cellulose acetate. 1 It has been isolated in maternal serum during pregnancy. 2 It has been isolated in metastatic placental to the plasmatic surfaces and in adeno-carcinoma of the pancreas.

PA ISOENZYME: A rare isoenzyme observed in sera of patients with pancreatic cancer. 19 Chab observed the band in 16 patients (15 with carcinoma of the pancreas and one with cholangiocarcinoma). ULTRA-FAST BAND: A band migrating in the albumin position on celulose acetate. 1" Controversy exists as to the identification of this band. It may be an artifact caused by an albumin–bilirubin complex or some other substance, or in some instances, it may be a true protein. Further research must be conducted to determine its true origin and significance.
The Helena Alkaline Phosphatase Isoenzyme Procedure is intended for the qualitative and/or quantitative determination of serum alkaline phosphatase isoenzymes by electrophoresis on cellulose acetate.

**SUMMARY**

Alkaline phosphatase (ALP) (EC 3.1.3.1.) is an enzyme which catalyzes the hydrolysis of phosphate esters at an alkaline pH. The greatest concentrations of ALP are found in bone, liver, intestine, and the placenta. However, practically every body tissue contains at least a small amount of ALP. Because of this wide distribution, limited information can be obtained from a total ALP assay. Fortunately each source of ALP produces one predominant isoenzyme and the tissue source of elevated ALP in serum can be determined by identifying the isoenzyme. The isoenzymes of ALP differ in their physicochemical and electrophoretic properties and, by taking advantage of these differences, the individual isoenzymes can be identified. In addition to the liver, bone, intestinal and placental isoenzymes, other ALP isozymes have been described. These include fast liver (pre-liver), Regen, Naga, PA, and renal isoenzymes.

A number of laboratory procedures have been used for the routine evaluation of the ALP isoenzymes. These include heat inactivation, inhibition with amino acids, urea denaturation, and electrophoresis on agarose, paper, starch gel, polyacrylamide gel and cellulose acetate.

The Helena cellulose acetate method offers several distinct advantages over other identification methods. The technique is suitable for the qualitative and/or quantitative determination of serum alkaline phosphatase isoenzymes. The following materials are necessary for use in the ALP procedure:

- **Reagents**
  - Magnesium Chloride
  - 2-Amino-2-methyl-1-propanol
  - 3-5
di-iodo-2-(4-chlorophenyl)-1 H-imidazole
  - 2-aminophenol and Magnesium Chloride

- **Materials Provided**
  - Helena Marker 5000
  - Zip Prep 5090
  - Zip Zone® Chamber Wicks 5081
  - Glue Stick 5002
  - Titan Gel Chamber 4063
  - Titan Plus Power Supply 1054
  - Helena Blotter Pads 5050
  - Zip Prep 5090
  - Helena Marker 5000
  - Glue Stick 5002

**BIBLIOGRAPHY**