true isoenzyme. Further research must be conducted to determine its true origin and significance.

BIBLIOGRAPHY
Isolated from the sera of patients with
1, 3-5
Figure 1: Place 10 µL of sample into each
1.  Properly code the required number of Titan
A. Preparation of Titan
SUMMARY OF CONDITIONS
Glue Stick 5002
Zip Zone
Titan Blotter Pads 5037
Alkaline Phosphatase Indolyl Blue Reagent 5102
Alkaline Phosphatase Isoenzyme Control 5139
Titan
plates may be wetted by slowly and uni
Apply this loading to a piece of
using the Microdispenser. Cover
3.  Place the plate, cellulose acetate side up, aligning
the edge of the plate with the black
scribed line marked “CENTER APPLICATION”. The plate
should be positioned so that the identification mark is
always aligned with sample #1.
4.  Apply the sample to the plate by depressing the appli
the sample wells 3 or 4 times and promptly trans
ferring the applicator to the Alkaline Base. Press the button
down and hold it 5 minutes. Make 2 to 3 superimposed
voltage bands using the wick.
D. Electrophoresis of Sample Plate
1.  Quickly place the plate in the chamber cellulose acetate
side (wet surface). Place a wet glass slide, etc, on the plate to
insure contact with the wicks.
2.  Electrophoresis for 20 minutes at 180 volts.
E. Visualization of Isoenzyme Bands
1.  Remove the sample plate from the chamber at the end of
the electrophoresis period and blot lightly. Place the plate,
cellulose acetate side up, on the blotter. Pipette 1.5 mL of
the reagent onto the cellulose acetate surface. Tilt the blot
under the wick.
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 it 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 56°C oven for 10 minutes.
F. Evaluation of the ALP bands
Quantitative evaluation: The ALP plates may be inspected visually for the presence of the isoenzyme bands.

Quality Control: The Alkaline Phosphatase Isoenzym Control (Cat. No. 5139) verifies all phases of the procedure and should be used on each plate run. The control may be used as a mark for the proper location of the bands.

RESULTS

Evaluation 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 band migrates in the beta, gamma, region. Placental, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha, beta, region, but they appear as tighter bands than bone. PA migrates cathode to the isoenzyme with gamma band in gamma region.

An ultra-fast band which migrates in the albumin region is seen occasionally using the visualization method. It is believed to be caused by blubium bound to alkaline.56,7 A specimen containing both liver and bone may exhibit one wide diffuse band in the alpha, pre-beta region. When such a pattern is obtained, heat inactivation may be helpful in distinguishing the two isoenzymes.

Bone, being extremely heat sensitive, will be 90-100% inactivat
ed approximately 5 minutes after the sample is affected. See the section entitled Further Testing and Special Treatment of the Serum Specimen for instructions for heat inactivation. Heat inactivation may be performed on all serum samples containing bone and/or Nagao isoenzymes. These three isoenzymes are extreme
ly heat stable and will not be inactivated by heat at 56°C for 10 minutes.

Sample dilution: Specimens containing both bone and liver isoenzymes may exhibit one broad diffuse band in the alpha, pre-beta region because the bone and liver do not separate. Dysert57 has reported results in the separation of bone and liver isoenzymes by diluting serum specimens with saline (0.85%) so that the total ALP is no greater than 200 U. Both a diluted and non-diluted sample should be run side by side on the same cellulose acetate plate.

INTERPRETATION OF RESULTS
LIVER ISOENZYMES: The major liver isoenzyme (in the α position) is the isoenzyme most frequently elevated when total ALP levels are elevated.8 9 The liver ALP increases in the blood early in liver disease before other liver function tests show abnormalities. An extensive group of conditions lead to increased 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, prima
tary biliary cirrhosis, and metastatic carcinoma to the liver.

Fast liver (in the α position) has been isolated in cases of meta
stastic carcinoma to the liver and has been suggested as a diag
nostic tool in identifying such cases. It has also been isolated in patients with viral hepatitis, liver cysts, and other liver diseases. Data generated in a study by Viot and his associates10 suggest that α 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.

BONE ISOENZYMES: Elevated as a result of increased osteo
blastic activity. This isoenzyme is normally elevated in growing children and bone tissue of the femur. The highest ALP values have been attributed to an increased bone isoenzyme level due to bone growth or renal rickets. An abnormal high bone isoenzyme level may also be indicative of bone can
cer, osteomalacia or coeliac sprue. A decreased bone ALP in childhood may be indicative of osteomalacia or hypophosphatasia.

PLACENTAL ISOENZYME: Appears in the serum of pregnant women late in the first trimester of pregnancy and may remain elevated for one month after termination of pregnancy.11 Infarction of the placenta in toxemia increases the serum pla
cental isoenzyme.

INTESTINAL ISOENZYME: Normally seen in the serum of subjects who have B or O blood types, especially after a fatty meal. Pathologically, the band may be present in perforation of the small or large bowel, the presence of the intestine and family in liver cirrhosis, as well as in intestinal perforation.12

RENAL ISOENZYME: A rare isoenzyme reported by Norden and Knapp which, like the Regan isoenzyme, migrates to the placental position. This isoenzyme represents a disease state of the kidneys or resection of kidney transplant. Renal isoenzyme: Isolated from the sera of patients with liver, drug induced liver disease, obstruction of biliary flow by carcinoma, ovarian cancer, and carcinoma of the colon.

NAGAO ISOENZYME: A variant of Regan isoenzyme that migrates in the same position as Regan on cellulose acetate.13 It has been isolated in metastatic carcinoma to the pleural surfaces and in adi
noma of the liver, pancreas or bile duct.

PA isoenzym: An unusual band observed in sera of patients with pancreatic cancer.14 Cha observed the band in 16 patients (15 with cancer of the liver and one for hypothyroidism).

ULTRA-FAST-BAND: A band migrating in the albumin position on cellulolose acetate.15 Controversy exists as to the identification of this band. It may be an artifact caused by an albumin-bilirubin com
plex or by other substance, or in some instances, it may be a
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 the mark in a corner of the plate.
2. Dissolve one bag of Electro® HR Buffer in 750 mL deionized water.
3. The plates should be soaked in the Buffer for 30 minutes according to the instructions. Each of the wicking pads or plates may be wetted by slowly and uniformly lowering a rack of plates into the buffer. The samples may be manually soaked for soaking up to 12 plates, or for approximately one week if stored tightly closed. Imperfect storage may cause poor separation of the isoenzymes.

B. Preparation of Zip Zone® Chamber
1. Pour approximately 100 mL of buffer into the cellu-lose acetate electrophoresis plate. Should heat inactivation be required, sons. An abnormally high bone isoenzyme level may also be indicative of bone cancer, osteomalacia or colicai sprue. A decreased bone ALP in childhood may be indicative of osteogenesis imperfecta or hyopophosphatasia.

PLACENTAL ISENZYMES: Appears in the serum of pregnant women late in the first trimester of pregnancy and may remain elevated for one month after termination of pregnancy. 6 Infarction of the placenta in toxemia increases the serum placentals.

INTERSTINAL ISENZYMES: Normally seen in the serum of subjects who have B or O blood types, especially after a fatty meal. Pathologically, the band may be present in perforation of the bowel, bowel, ulcerative diseases of the intestine and faintly in liver cancer, as well as in intestinal perforation. 6

RENAL ISENZYMES: A rare isoenzyme reported by Nisenoff, R., et al. 7 The liver ALP increases in the pre-beta region of the serum albumin. The control may be used as a marker for the proper location of the bands.

RESULTS
The evaluation of the alkaline phosphatase isoenzyme migration are described in comparison to typical serum protein formations. Fast liver (pre-liver) migrates in the protein alpha, region and the major liver band migrates in the beta, region. Placental, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region. Nisenoff, Regan, Nagao, and renal isoenzymes migrate with bone in the alpha,beta region, but they appear as tighter bands than bone. PA mimics c-cathodo to the beta, region.

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BONE ISENZYMES: Elevated as a result of increased osteoelastic activity. This isoenzyme is normally elevated in growing children and of approximately 10% of the highest ALP values have been attributed to an increased bone isoenzyme level due to factors such as renal rickets. 5 An abnormality of this type is in the pre-beta region. The high bone isoenzyme level may also be indicative of bone cancer, osteomalacia or colicai sprue. A decreased bone ALP in childhood may be indicative of osteogenesis imperfecta or hypophosphatasia.

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NAGAO ISENZYMES: A variant of Regan isoenzyme that migrates in the same position as Regan on cellulose acetate. 1, 8 It has been isolated in metastatic carcinoma to the pleural surfaces and in addition, in pleural fluid and bile duct.

PA ISENZYMES: An unusual band observed in sera of patients with pancreatic cancer. 10 Chia observed the band in 16 patients (15 with cystadenocarcinomas and one with adenocarcinomatous).

ULTRA-FAST BAND: A band migrating in the albumin position on cellulose acetate electrophoresis. 1, 9 The band consists of two to three bands consistently. If the band is present, it is suggestive of either metastatic disease or malignancy. It may be indicative of a malignancy, but the band may be a composite or by some other substance, or in some instances, it may be a false positive.
true isoenzyme. Further research must be conducted to determine its true origin and significance.


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The Helena Alkaline Phosphatase Isoenzyme Procedure is intended for the qualitative 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 plasma isoenzymes, other ALP isoenzymes have been identified in serum. These include fast liver (pre-liver), Regen, Nagao, 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 suitabe for the electrophoretic fractionation of ALP in large numbers of sera, provides ease of handling, stability, reproducibility of results.

PRINCIPLE

The isoenzymes of alkaline phosphate are separated according to their electrophoretic mobility on cellulose acetate in a tris-barbitol-sodium barbitol buffer. The colorimetric reaction occurs by hydrolyzing the indolyl dye.

REAGENTS

1. Alkaline Phosphatase Indoly1 Blue Reagent (Cat. No. 5102) Ingredients: When reconstituted as directed, the concentration of the reactive ingredients is as follows:

- 5-Bromo-3-indoly1 Phospho-p-Toluidine Salt
- 2-Amino-2-methyl-1-propanol
- Magnesium Chloride

**Stabilizers**

**WARNING: FOR-VITRO DIAGNOSTIC USE. DO NOT INGEST.**

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

Storage and Stability: The reagents should be stored at 2 to 8°C and are stable until the expiration date indicated on the vial.

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

2. Alkaline Phosphatase Indoly1 Blue Diluent Ingredients: When reconstituted as directed, the concentration of the reactive ingredients is as follows:

- 2-Amino-2-methyl-1-propanol and Magnesium Chloride

**WARNING: FOR-VITRO DIAGNOSTIC USE. DO NOT INGEST.**

Preparation for Use: The diluent is ready for use as packaged.

Storage and Stability: The diluent should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.

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

3. Electra® HR Buffer (Cat. No. 5805) Ingredients: The buffer is a tris-barbital-sodium barbitol buffered at an alkaline pH. **WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST.**

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 packaged 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 shows signs of dampness or discoloration. 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.

**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 ALP above 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 competing 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.

**INTERFERING SUBSTANCES**

**PROCEDURE**

**MATERIALS PROVIDED**

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

**Item**

<table>
<thead>
<tr>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>Super Z-12 Applicator</td>
</tr>
<tr>
<td>Super Z-12 Sample Well Plate (2)</td>
</tr>
<tr>
<td>Super CPK Aligning Base</td>
</tr>
<tr>
<td>Titan Gel Chamber</td>
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<tr>
<td>Diaminished reagent and Tubes</td>
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<tr>
<td>1000 Staining Set</td>
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<td>Development Weight</td>
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