Laboratory Procedure Manual

Analyte: **Total Iron Binding Capacity (TIBC)**

Matrix: **Serum**

Method: **Beckman Synchron LX20**

Method No.:

Revised:

as performed by: *Collaborative Laboratory Services*
Ottumwa, Iowa

Contact:

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Important Information for Users

Collaborative Laboratory Services periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
FETIB_D	LBXTIB	TIBC, frozen serum (µg/dL)
	LBDTIBSI	TIBC, frozen serum (µmol/dL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Total Iron Binding Capacity (TIBC) is done indirectly by the Unsaturated Iron Binding Capacity (UIBC) method.

A known ferrous iron standard 105 µmol/L (586 µg/dL) incubated with serum at a pH of 7.9 saturates the available binding sites on serum transferrin. The unbound excess iron is then complexed with ferene® to form ferrous ferene, a blue complex, which is measured by the LX system. The UIBC is equal to the total iron added less the excess iron.

In 1970, Stookey reported the synthesis of Ferrozine which complexed with ferrous iron to form a tris ferrozine/iron, $Fe(Fz)_3$ complex. The major advantages of ferrozine are the high molar absorptivity of the ferrous ferrozine complex (28,000), its water solubility, and stability over the pH range of 4–9. This assay uses a ferroin type compound, Ferene. This reagent is a superior iron chelating agent forming a Ferene complex with ferrous iron with a maximum absorbance at 593 nm and a molar absorptivity of 35,500. LX20 uses 600 nm analytic wavelengths. The compound has a 27% higher molar absorption than ferrozine, absorbs at a longer wavelength, and has the other advantages of ferrozine, namely its solubility and stability.

UIBC measurements (in conjunction with serum iron measurements) are used in the diagnosis and treatment of hereditary hemochromatosis and other iron disorders. The combined value of UIBC and serum iron gives a value for the TIBC. This represents the maximum concentration of iron that serum proteins can bind.

TIBC is elevated in iron deficiency. The sensitivity of TIBC for iron deficiency is less than that of serum ferritin. TIBC tends to be low in anemia of chronic disease. Again predictive value is not high and low TIBC is interpreted with ferritin and hematologic parameters.

Iron Method

The method used to measure the iron concentration is a timed-endpoint method. In the reaction, iron is released from transferrin by acetic acid and is reduced to the ferrous state by hydroxylamine and thioglycolate. The ferrous ion is immediately complexed with the FerroZine Iron Reagent. The system monitors the change in absorbance at 560 nm at a fixed-time interval. This change in absorbance is directly proportional to the concentration of iron in the sample.

Serum iron measurements in conjunction with total iron binding capacity are useful in the diagnosis and treatment of disorders relating to iron intake, absorption, storage, and release mechanisms. Such changes are indicative of a wide range of dysfunctions including anemias, nephrosis, cirrhosis, and hepatitis.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves and laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact plasma and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Sani-Cloth HB, Germicidal Disposable Wipe when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

A. Microsoft Excel software on a PC and our Laboratory Information Systems (L.I.S.) are used to manage the data. The test is analyzed on a Beckman Synchron LX20. When all ordered tests are completed for each sample, the results are printed out by a Beckman Synchron LX20 instrument.

The LX20 is interfaced to the L.I.S. with a bi-directional interface. After tests are completed, the results will go to the L.I.S. Host Computer Interface to be verified by qualified analyst.

B. Reflex testing is set up in the L.I.S. to order a repeat of any critical result, to verify abnormal values.

C. Statistical evaluations of the runs are accomplished with Microsoft Excel software on a PC.

D. The file is opened and copied to an Excel spreadsheet for evaluation. The Excel spreadsheet results file data are copied to the shipment file and sent using Internet FTP transfer of file or e-mailed to Westat within 21 days of sample receipt.

E. The Excel files containing all raw data and results are backed up once a week using a CD writer or External Hard drive for storage. Files stored on the L.I.S. network are automatically backed up nightly to tape.

F. Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. UIBC

(1) Interferences:
  (a) No interference from bilirubin or lipemia.
  (b) Do not use hemolyzed specimens.
(2) Fasting is recommended.
(3) A minimum of 0. 3 mL of serum is needed for the UIBC analysis.
(4) Sample volume for individual test is 15 µL added to 225 µL of UIBC reagent.
(5) Sample is run in duplicate for this study.

B. Iron

(1) Interferences:
  (a) No interference from lipemia or bilirubin <30 mg/dL.
  (b) No interference from hemolysis.
(2) Serum/plasma should be separated from the red cells within two hours after collection.
(3) Separated serum or plasma should not remain at +15 to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2 to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at –15 to –20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.
(4) Samples should be drawn in the morning due to diurnal variation.
(5) Iron-dextran administration can cause elevations or iron with this method.
(6) Ingestion of oral contraceptives will elevate iron or TIBC values.
(7) A minimum of 0.3 mL of serum is needed for the Iron analysis.
(8) Sample volume for individual test is 25 µl added to 210 µl of iron reagent.
(9) Sample is run in duplicate for this study.
(10) Only plastic or borosilicate glass containers should be used to store samples.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Instrumentation: Beckman Synchron LX20

B. Materials

(1) Beckman Synchron CX Micro Sample Tube (Part #448774)
(2) S/P Plastic Transfer Pipette (Cat. #P5214-10)
(3) S/P Brand Accutube Flange Caps (Cat. #T1226-37)

C. UIBC

(1) Reagent Preparation: DCL UIBC Reagent purchased from Beckman Coulter.
(a) Diagnostic Chemicals Limited (UIBC Reagent Kit (Cat. #153-10 or Beckman Part #475949).
(i) R1: Binding Buffer Reagent (1 x 100 mL)
(ii) R2: Iron Color Reagent (1 x 25 mL)
(b) Reagent Preparation: Reagents as provided are ready to use.
(i) Into Compartment “A” of a Beckman user-defined reagent cartridge, pour 33 mL of R1 (Binding Buffer). Measure the 33 mL with a graduated cylinder so there is enough R1 reagent in each kit to make up 3 user-defined cartridges.
(ii) Into Compartment “B” of the user-defined cartridge, pour 8 mL of R2 (Color Reagent). Accurately measure the 8 mL so there is enough R2 reagent for 3 cartridges.
(iii) Compartment “C” will be empty.
(iv) Load cartridge onto instrument using the Manual Load instructions in the LX20 Operating Procedure.
(c) The reagents, as provided, are stable until expiration date when stored at 2–8°C. UIBC reagent is stable on board for 56 hours. The iron color reagent should be protected from light.

(2) Standards Preparation:
(a) Diagnostic Chemicals, Ltd. UIBC-Cal (Catalog #SE-090).
(b) Reconstitute with 10 mL of deioinized water. Mix gently and allow to stand for 15 minutes, swirling occasionally.
(c) unopened vials are stable until expiration date when stored at 2–8°C.
(d) Reconstituted calibrator is stable for two weeks when stored at 2–8°C.
(e) Reconstituted calibrator should be brought to room temperature prior to use.

(3) Control Material:
(a) Diagnostic Chemicals, Ltd. DC-Lineate UIBC in Tri Level Calibration/Linearity Material (Catalog #SE-091).
(i) DC-Lineate UIBC values are referenced to National Institute of Standards and Technology (NIST) material.
(ii) Reconstitute each level (1, 2, 3) with 5 mL of deionized water. Mix gently and allow to stand for 20 minutes, swirling occasionally until dissolved.
(iii) Unopened vials are stable until expiration date when stored at 2–8°C.
(iv) Reconstituted lineate is stable for 14 days when stored at 2–8°C.

(v) Reconstituted lineate should be brought to room temperature prior to use.

(b) Pooled serum prepared at CLS aliquoted and frozen.

B. IRON

(1) Reagent Preparation: Beckman Synchron Systems Iron Reagent (*Part #467910).

(a) No preparation required.

(b) When stored unopened at 2–8°C, the reagent is stable until the expiration printed on the label.

(c) When first opened or installed on the instrument, the reagent is stable for 60 days unless the expiration date is exceeded.

(d) Do not freeze.

(2) Standards Preparation: No preparation required.

(a) Synchron Iron/TIBC Calibrator Kit (Part #442772).

(3) Control Material

(a) Beckman Triad Custom Unassayed Chemistry Control Serum (Part #465405).

(b) Pooled serum prepared at CLS aliquoted and frozen.

(4) Use disposable labware whenever possible. Rinse glassware with 0.1 N HCl before use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. UIBC

(1) Calibrators: Diagnostic Chemicals UIBC-CAL (Catalog #SE-090)

(a) One level

(b) Note: Deionized water is used at a 0.0 calibration point.

(c) NOTE: Assigned calibrator values are lot-specific. DO NOT interchange lots of calibrator.

(2) Calibration: Perform calibration according to Calibration Procedure in LX20 Operation Procedures.

(a) Calibrator 1 = Type I deionized water (Calibrator Code UIBC1).

(b) Calibrator 2 = DC-UIBC-CAL (Calibrator Code UIBC2).

(c) Calibration frequency: Daily.

B. IRON

(1) Calibrators: Synchron Iron/TIBC Calibrator Kit (Part #442772)

(a) Store at ambient temperature.

(b) Calibrators are stable until expiration date on bottle when stored at ambient temperature.

(c) The bottles of calibrator solutions should be kept tightly closed, except when drops are being dispensed. Evaporation and/or contamination will result in erroneous assay values.

(d) Caution: The calibrator solutions contain HCl, pH<2, which is corrosive. Do not pipette by mouth. Protective clothing, gloves, and safety glasses should be worn when handled this product. Avoid contact with eyes and skin. If spilled, flush with water.

(2) Calibration frequency:

(a) 14 days for each individual reagent cartridge.

(b) Note: Within-lot calibration is not available for iron.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

Enter test in L.I.S. according to procedure for Iron and TIBC listed in this document.

B. Sample Preparation

Procedure for labeling CX sample tubes for Iron and TIBC and transferring serum (See Attachment E).

C. Operation

(1) Refer to Operation Procedures for programming controls/patients and loading sectors/racks in the Beckman LX20 Chemistry Information Manual, 2001 (See Attachment F for specific procedure for NHANES samples).

(2) Duplicate UIBC samples are repeated if the difference between duplicates is greater than 10%. The repeat values are used if repeats are within 10%. Investigation and new aliquots are assayed if repeat values are not within 10%.

D. Recording of Data

(1) Operator will review and verify results in the L.I.S.

(2) Operator will place printouts in box labeled for NHANES samples.

(3) The L.I.S. reorders tests to verify any critical results. These results are stored in the L.I.S. along with the original results. Original values are used when repeat results match the original within 3 CV.

(4) Project supervisor will export data from the L.I.S. into an Excel file. The data is copied into another Excel file for further evaluation.

(5) A printout of the Excel spreadsheet for each container ID results is made and comments noted.

(6) Project supervisor reviews the results. If problems noted with patient results or QC, Project Supervisor investigates and discusses issues if necessary with Laboratory Director. Repeat samples if necessary.

(7) Daily log sheets are completed and any problems or issues noted.

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E. Replacement and Periodic Maintenance of Key Components

F. (See Attachment AB for LX20 Maintenance Schedule).

G. Calculations:

H. Synchron LX Systems perform all calculations internally to produce the final UIBC result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

I. TIBC = UIBC + Serum Iron.

J. The L.I.S. calculates TIBC and percent saturation when ordered with Iron. TIBC results are averaged in Excel and the average TIBC is reported to NHANES. The average Iron is also reported.

9. REPORTABLE RANGE OF RESULTS

A. UIBC Analytical Range:

(1) 0-500 µg/dL.

(2) If results print “RESULT SUPPRESSED OIRL”, report UIBC as “<20”. If results are over the linearity limit or “RESULT SUPPRESSED OIRH”, dilute with saline and reanalyze.

(3) Limits of detection (LOD) are established by Beckman Coulter and linearity data verifies the reportable range. Detection of results below the reportable range is not relevant and formal limit of detection study is unnecessary.

(4) Sensitivity is defined as the lowest measurable concentration. Sensitivity for the UIBC determination is 20 µg/dL.

(5) 0 is not a reportable value.

B. Iron Analytical Range:

(1) 5–500 µg/dL.

(a) On samples which are out of instrument range high, repeat test on a dilution of the sample with saline.

(b) then the test is repeated on a dilution, the dilution factor must be entered at the Sample Information Screen.

(c) If the dilution factor is not entered into the instrument, the printout value must be multiplied by the dilution factor to obtain the final result.

(2) Limits of detection (LOD) are established by Beckman Coulter and linearity data verifies the reportable range. Detection of results below the reportable range is not relevant and formal limit of detection study is unnecessary.

(3) Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the iron determination is 5 µg/dL.

(4) Samples which are out of instrument range low should be reported as “5 µg/dL”.

(5) 0 is not a reportable value.

(6) Repeat all irons <5 µg/dL before reporting.

10. QUALITY CONTROL (QC) PROCEDURES

A. Blind QC Specimens are included in the samples received from NHANES.

B. DC-Lineate levels 1, 2, and 3 are assayed before CDC-NHANES samples. Pooled serum is run in the middle of running samples. DC-Lineate levels 1, 2, and 3 are again assayed after complete testing CDC-NHANES samples.

C. Beckman Triad Custom Unassayed Chemistry Controls Levels 2 and 3 are assayed for Iron in early A.M. and if a new reagent pack is loaded, controls are assayed again. One level is assayed in middle of the day and both control levels are assayed after running NHANES sample.

D. Pooled serum is run on UIBC and Iron in the middle of running samples.

E. Acceptable Answer:

(1) Controls must be within ±2 SDs.

(2) Refer to Quality Control Flow Chart for action decisions guidelines (See Attachment I).

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. UIBC

(1) Hemolysis >3+ demonstrate a negative interference.

(2) Bilirubin has no significant interference.

(3) Lipemia has no significant interference.

(4) Copper is the only cation of the trace metals usually present in serum to form a colored complex with ferene. Copper interference with ferene is similar to that encountered with ferrozine and studied by Duffy and Gaudin. Ninety-five percent of the copper interference is eliminated by chelation of free copper.

(5) Refer to References for other interferences caused by drugs, disease and preanalytical variables.

B. IRON

(1) Do not use hemolyzed sample. Hemoglobin levels of 60 mg/dL (1+) will elevate Iron results by 12 µg/dL.

(2) Bilirubin 30 mg/dL has no significant interference.

(3) Lipemia has no significant interference.

(4) Copper and magnesium have no significant interference.

(5) Iron-dextran administration can cause elevations of iron with this method.

(6) Ingestion of oral contraceptives will elevate iron or TIBC values.

(7) Refer to References for other interferences caused by drugs, disease and preanalytical variables.

13. REFERENCE RANGES (NORMAL VALUES)

Serum	µg/dL
TIBC	250–450
Iron – Male	50–160
Iron – Female	40–150

Reference Range values were established from wellness participants with an age mix similar to our patients. These data were analyzed using non-parametric techniques described by Reed (Clin Chem 1971;17:275) and Herrara (J Lab Clin Med 1958;52:34-42) which are summarized in recent editions of Tietz' textbook. Descriptions appear in Clin Chem 1988;34:1447 and Clinics in Laboratory Medicine June 1993;13:481.

Pediatric Reference Range Guidelines for Synchron Systems- Multicenter study using data from Montreal, Quebec, Miami, FL and Denver, CO. Beckman 1995.

14. CRITICAL CALL RESULTS (PANIC VALUES)

There are no critical call back values for TIBC or Iron.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive frozen on dry ice. Specimens are kept in –70°C freezer until ready to transfer to CX multi sample tubes. Samples are thawed, mixed well, and then transferred to CX multi sample tubes. Capped CX sample tubes are kept refrigerated until ready to put on instrument.

Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of pour off tubes. Specimen vial container is placed in –70°C Freezer after testing is complete. CX sample tubes are refrigerated, then frozen after analysis.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

    Samples will remain in –70°C freezer until instrument is back in operation.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

    Test results are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, either through Internet FTP transfer of files or electronic mail or other electronic means.

    All data are reported electronically to Westat within 21 days of receipt of specimens.

    Internet FTP transfers of files or dial up modem transfer options are available.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

    In general, when specimens are received, the specimen ID number, and a name identifying the container ID and slot number is entered into the Laboratory Information System (L.I.S.) database. New barcodes are printed and the specimens stored in a refrigerator. Samples are aliquoted to a CX-Micro Sample tube with the new barcodes. The specimen ID is read off of the tube by a barcode reader. Tracked in the database are the date and time of entry into the L.I.S., date and time analysis completed, and who certified the results.

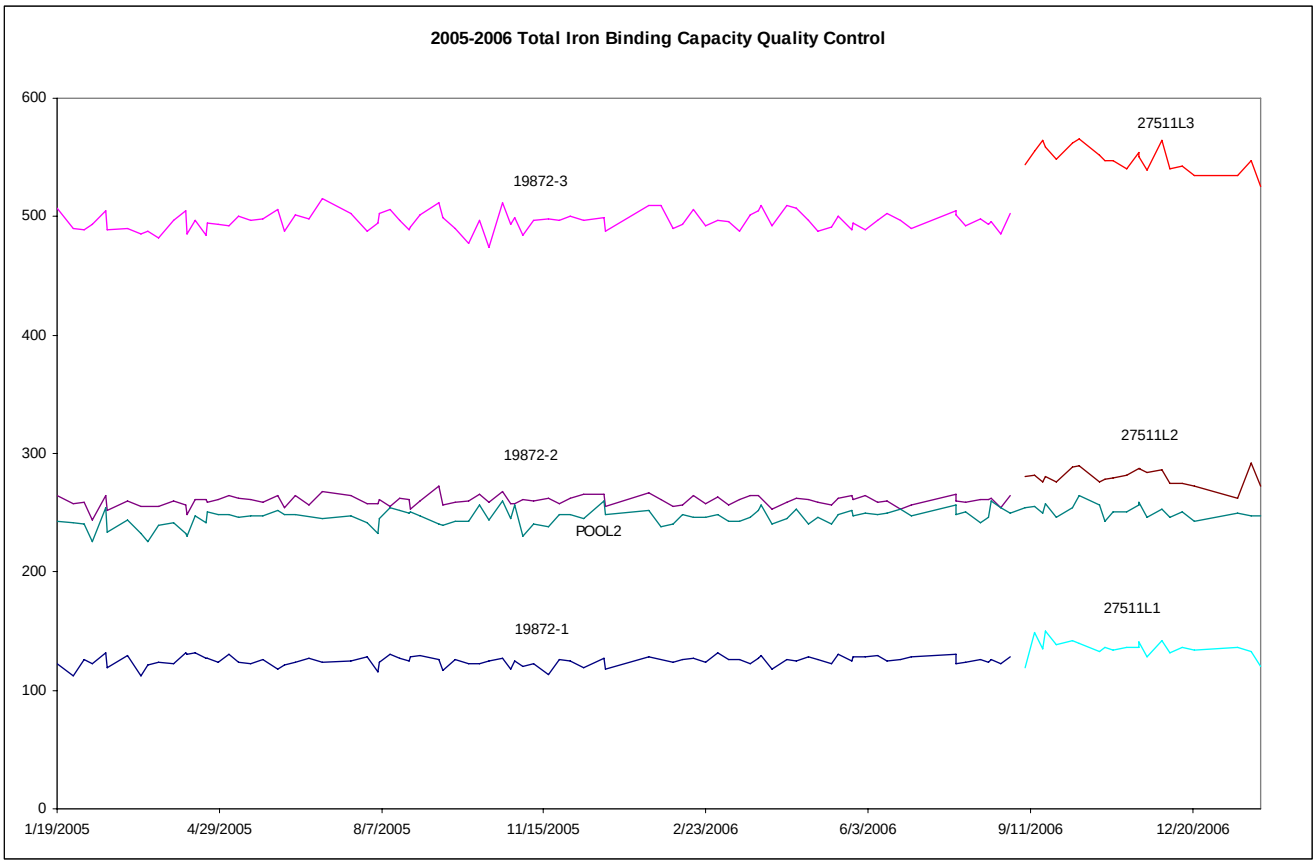
    Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

    The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Total Iron Binding Capacity by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
19872	86	1/19/2005	8/30/2006	124.8	4.3	3.5
POOL2	106	1/19/2005	1/31/2007	246.9	7.2	2.9
19872-2	86	1/19/2005	8/30/2006	260.0	4.5	1.7
19872-3	86	1/19/2005	8/30/2006	496.3	8.0	1.6
27511L1	21	9/8/2006	1/31/2007	135.7	7.5	5.5
27511L2	21	9/8/2006	1/31/2007	280.2	7.2	2.6
27511L3	21	9/8/2006	1/31/2007	548.6	10.9	2.0



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