Good judgment comes from experience, and experience comes from bad judgment.

Materials & Methods
D. MATERIALS AND METHODS

The study was carried out at the Sterling Hospital, Ahmedabad, India. Good Clinical Practices were followed upon as per ICMR and ICH guidelines. The protocol was approved by the Institutional Ethics Committee. All the participants provided written informed consent before participation in the study. Case Report Forms were filled for the patients. Baseline demographic details as well as biochemical parameters were collected. Patient's history for diabetes, hyperlipidemia, hypertension, CAD etc. was also recorded.

D.1 Study Population

D.1.1 Inclusion Criteria

1. Patients of either sex ≥ 18 years of age
2. Established diagnosis of hyperlipidemia with fasting LDL-C levels ≥ 130 mg/dl
3. Normal routine hematological and biochemical test results

D.1.2 Exclusion Criteria

1. Pregnancy & lactation
2. Patients with acute liver disease or hepatic dysfunction indicated by AST or ALT levels ≥ 2.5 times the upper limit of normal
3. Patients with history of myopathy or evidence of active muscle diseases
4. Patients on concomitant medications known to affect lipid levels
5. Patients with any other serious concurrent illness or malignancy
6. Patients with continuing history of alcohol and / or drug abuse
D.1.3 Withdrawal Criteria

1. The patient suffers from significant intercurrent illness
2. Any patient found to have entered the study in violation to this protocol
3. Any patient who requires the use of an unacceptable concomitant medication
4. Any patient who interrupts the study medication
5. If it is felt in Investigator’s / Medical Expert’s opinion that it is not in the patient’s best interest to continue
6. Any patient / relative who wishes to withdraw his / her consent for participation in the study

D.2 Study Design

156 Patients were divided into three different groups, 52 patients in each group: Group 1: Patients taking ezetimibe 10 mg; Group 2: Patients taking atorvastatin 10 mg; Group 3: Patients taking ezetimibe 10 mg plus atorvastatin 10 mg (ezetimibe+atorvastatin) (Purity of study drugs were 95% to 105%). They were assigned as per physician’s advice. All doses were administered for 52 weeks orally. Patients also remained on the lipid-altering diet throughout the treatment period. If the patient was taking other lipid lowering medications in initial phase, study drug was started after the wash-out.

D.3 End Point

D.3.1 Efficacy Endpoints

The primary efficacy endpoint was per cent change in LDL-C, from baseline to study endpoint after treatment with ezetimibe versus atorvastatin versus ezetimibe+atorvastatin. The endpoint value is
defined as the last post-baseline measurement done during the 52 weeks period. Secondary efficacy endpoints included per cent change in other lipid and lipoprotein parameters (TC, TG, HDL-C) from baseline to study endpoint.

**D.3.2 Safety Endpoints**

Safety and tolerability were assessed by clinical and statistical reviews of all safety parameters, including adverse events (AEs), laboratory values, and vital signs. All AEs were rated by the investigators as definitely not, probably not, possibly, probably, or definitely related to treatment. AEs of clinical interest included patients experiencing: consecutive elevations of CK >10×ULN without muscle symptoms or >5×ULN with muscle symptoms; myopathy (muscle symptoms accompanied by CK >10×ULN); and elevated hepatic transaminases (consecutive 3×ULN elevations in ALT and/or AST).

**D.4 Blood Collection and Sample Analysis**

Blood samples were collected after a 12 h fast at the baseline and at Weeks 52. Complete lipid profiles and non-lipid parameters were measured in plasma at baseline and at week 52. These variables included total cholesterol (TC), direct LDL-C, triglyceride (TG), HDL-C, high Sensitivity c-reactive protein (hS-CRP), creatine kinase (CK), homocysteine, Lp(a), SGOT, SGPT, alkaline phosphatase, creatinine, urea, uric acid. All parameters were measured directly with preparative ultracentrifugation and enzymatically with the Vitros 350.
(Johnson & Johnson). All clinical laboratory analyses were performed at the Sterling hospital, Ahmedabad, India.

D.5 Statistical Analysis

Baseline data were expressed as Mean±SEM (Standard Error of Mean). End point data represented as percentage change from baseline. All data were analyzed by using paired t-test using SigmaStat 2.03 software. Data were considered significant only when p<0.05. 52 patients data in each group were considered for statistical analysis.
D.6 Instrument

Vitros 350 Chemistry System Components

Designed with "a lot of power in a convenient package," the Vitros 350 Chemistry System runs up to 350 tests an hour. It processes 40+ direct tests, using different chemistry slides, and can calculate 14 derived tests.

The Vitros 350 System has two main components:

- The Main Unit
- The Control Unit

Main Unit

The main unit is the largest unit of the analyzer. It contains the computer system and the instrument modules for handling and processing slides.

Control Unit

The control unit is located to the right of the main unit. It consists of a display unit and a keyboard. The control unit serves as the primary interface between operator and analyzer. All major functions are initiated and monitored at the control unit.
Monitor Screen

The monitor screen is touch-sensitive, meaning you can access and monitor analyzer functions and input information by simply touching the screen's surface.

Output Devices

Printer can be attached as the output device for patient and calibration results. Test results can also be sent to a laboratory computer as an additional output device.

Computer Systems

Three sets of computers control all functions of the analyzer:

- The Master Computer
- The Scheduler Computer
- The Subsystems Computers

Master Computer

The master computer generally deals with the control unit and any external control-system components, such as the printer. The master
computer also coordinates activities between itself and the scheduler computer.

**Scheduler Computer**

The scheduler computer is really two computers that drive all of the mechanical and thermal components within the analyzer. It communicates with the various subsystem computers to coordinate and synchronize these activities.

**Subsystems Computers**

The subsystems computers each control a specific electromechanical function; for example, the incubator rotor or slide-supply environment.

**Sample Handler**

The left side of the analyzer shows hardware modules and instruments that handle patient samples. The diagram illustrates the terminology for and location of these modules within the analyzer.

**Calibration Theory**

In a simple linear system, calibration is performed by measuring the response (R) of standards at a high (Cs) and low or zero (Co) concentration. Unknown concentrations (Ca) can then be determined by interpolating from the calibration line between the two standards. In order for this type of calibration to be valid, the response between the two standards must be known to be linear.
Two methods where the measured response is not linearly related to analyte concentrations. We can compare the calibration of solution spectrophotometry methods, transmission method, to that of the Vitros 350 Chemistry System's dry-slide technology, reflection method.

![Graph showing response vs. concentration]

**Step 1**

**Transmission Method**

In solution chemistries, the incident light (Io) enters the solution at 100% level. The colored solution absorbs a certain percent of the light and the remainder is passed through and out of the solution. Therefore, an observer would see less light leaving the material than has entered it. The transmission method measures the light transmitted through the material. This transmitted light is measured (It):
Reflection Method

In dry slides, the incident light (IO) enters the slide at 100% level. The colored complex absorbs a certain percent of the light and the remainder is reflected. The reflection method therefore measures the light reflected. The reflectance measured is a specific portion of the total reflected (IR):

Step 2

Transmission Method

Transmission density ($C_T$), or absorbence, is determined by taking the logarithm of the reciprocal of $T$: 
Reflection Method

Reflection density ($DR$) is determined by taking the logarithm of the reciprocal of $R$:

$$DR = \log_{10} \frac{1}{R}$$

Step 3

Transmission Method

Following Beer's law, analyte concentration is directly proportional to $DT$: This relationship is linear and thus, predictable.

$$C = A_0 + A_1 \times DT$$

Reflection Method

With reflection, analyte concentration is not directly proportional to $DR$. Although this relationship is non-linear, it is predictable.
Light Scatter

The reason reflection density is non-linear is that it includes both reflected and scattered light. Within the slide, light is reflected and scattered as it hits the layers and surfaces. The photodetector measures a specific portion of this reflected light.
Vitros 350 Chemistry system

Specifications

Measurement Principles: Potentiometric (direct ISE), Colormetric/Rate, Immuno-rate

On-Board Test Capacity: 3600 tests

Sample Types: Serum, Plasma, Urine, CSF

Sample Volume: 5-11 μL

Sample Capacity: 40 samples (4 trays of 10 samples)

Time for Single Results: ~ 2.5 to 8 minutes

Potentiometric: ~ 2.5 minutes

Colormetric: ~ 5 minutes

Immuno-rate: ~ 8 minutes

Sample Containers: 10mL, 7mL, 5mL, 2-4mL Collection Tubes, 1mL Microtube, Microsample cup and .5mL and 2mL

Sample Management: Clot Detection, Bubble Detection, Liquid Level-Sensing (Pressure Transducer), Short-Sample Detection

Automated Technology (optional): Includes automatic tip loading and deep tube sampling

Calibration: Extended; multiple lots supported