APPENDIX - I

EPIDEMIOLOGY QUESTIONNAIRE

Date:
O.P No:  
Code No:

Age:

Blood Collection Date:

Repeated Blood Collection date (if any):

Blood Group Age:

Sex:

Occupation:

Duration of the ailment:

Degree of the ailment:

Medication taken:

Height:

Weight:

Caste:

DIETARY HABITS:

1. Vegetarian: Yes / No

2. Non-Vegetarian: Yes / No

3. Frequency of Intake
   Daily:
   Weekly:
   Monthly:
4. Junk Food

5. Frequency of Intake
   - Daily :
   - Weekly :
   - Monthly :

6. Do you eat Fried Food?: Yes / No

7. If yes, Which oil or Fat do you Fry in?

8. Type of cooking oil used:

9. Do you eat Snack Chips?

10. Do you put butter on your Vegetables?

11. Do you use a lot of Salad dressing?

MEDICAL AND GENETIC HISTORY:

1. Affected with Diabetes? Yes/No

2. Having Hypertension? Yes/No

3. Any case existing in Family or Relatives?

4. Have you ever had any type of Cancer? Yes/No

Reproductive History:

1. Age at Menarche

2. Regular menstrual cycle: Yes/No

3. Age at marriage

4. Have you ever used Oral Contraceptive Pill?: Yes/No

5. Age at first child birth:

6. Number of pregnancies:
7. Parity:

8. Nulliparity:

9. Number of abortions:

10. Age at menopause:

APPENDIX II
PATIENT DETAILS

O.P No Code No: .................................................................
Name: ..................................................................................
Age ....................................................................................
Blood collection date: ..........................................................
Repeat blood collection date (if any): .................................
Blood group: ........................................................................
Address: ............................................................................
Duration of the ailment: .....................................................
Degree of the ailment: ....................................................... 
Medication taken: .............................................................
Caste: ................................................................................
Marital status: - Single........................................ / Double ...........
Consanguinity (any inter-relation marriage): ........................
Paternal ............................................................................
Maternal ............................................................................
Eating habits: Vegetarian ...................................................
Non vegetarian - Frequency of intake: ..............................
   Daily ............................................................................
   Weekly ........................................................................
   Monthly ......................................................................
Junk food - Frequency of intake:
Daily ........................................................................
Weekly .................................................................
Monthly ..............................................................

Any hereditary disease: .....................................................
Any case existing in relatives family: ..............................
Any specific habit: Tobacco chewing ..............................
Pan ............................................................................
Any other ..................................................................
Duration .....................................................................

Occupational Details:
What kind of job have you held during last 10 years? Have you been exposed to dust or any other etiological factor while working there?

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<th>Position</th>
<th>Factors</th>
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Present Occupation:
Are you exposed to dust or any etiological factor while working there?

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Menstrual cycle ........................................................................
Regular / Irregular ................................................................
Normal/Scanty / Profuse .................................................
Reproductive history ........................................................
Use of contraceptives ........................................................
Number of still-born .........................................................
Number of abortions ........................................................
Number of children ........................................................
Medical history: Any chronic systemic disease –
  Tuberculosis ......................................................
  Diabetes ...........................................................
  Hyper-tension ....................................................
  Any other ...........................................................

Any acute viral infection for last 1 months ....................................................

Treatment taken:
  History of medication in last 3 months:.................................
  History of any X-ray taken before: ........................................

Miscellaneous : ................................................................

  ..................................................................................

APPENDIX III

PROPERTIES AND GENOTYPES OF THE TESTER STRAINS OF *Salmonella typhimurium*

In addition to the histidine mutation, the standard tester strains contain other mutations that greatly increase the sensitivity in detecting the mutagens. The *rfa* mutation cause partial loss of the lipopolysaccharide barrier that coats the surface of the bacteria, thus increasing the permeability of large molecules such as benzo(a)pyrene (BaP), that do not penetrate the normal cell wall. The other mutation, *(uvr B mutation)* involves the deletion of a gene coding for the DNA excision repair system, resulting in greatly increased sensitivity in detecting many mutagens (Ames, 1971).

TAI00 and T A 98 have been developed by transferring a resistance transfer factor (R factor) to the standard tester strains T A 1335 and T A 1538 respectively. These two new strains (TA 100 and TA 98) are extremely sensitive in detecting a number of mutagens and are recommended for use in general mutagenesis testing. (Maron and Ames, 1983).

Maintenance of tester strains
Upon receiving the strains, they were inoculated into nutrient broth (8 g nutrient broth, 5g NaCl, and 1000 ml distilled water) and allowed to grow at 37°C. The genotypes of the tester strains were confirmed as described below and the culture streaked on nutrient agar plates (Master plates).

Confirming genotypes of tester strains

**Histidine requirements**

The Histidine character of the tester strains was confirmed by demonstrating the histidine requirement for growth on selective agar plates (Biotin is also required by all the standard tester strains because of the uvr B deletion which extends through the biogene).

Each plate (with 0.1 ml of 0.5 mM Biotin and with or without 0.1 ml of O.1M Histidine) was streaked with the strains, incubated overnight at 37°C and examined for growth. The histidine requirements were shown by growth observed only in His/bio plate but not in the control plate with Biotin alone.

**rfa mutation**

The presence for rfa mutation was checked by testing the permeability of large molecules. Crystal violet was used for this purpose. Sterile filter paper disc, onto which 10ulf 1 of crystal violet solution (1mg/ml) has been delivered, was carefully placed on the solidified top agar to which the bacterial culture has been added. After overnight incubation at 37°C, a clear zone of inhibition was observed around the disc, indicating the presence of rfa mutation, permitting large molecules like crystal violet to enter and kill the bacteria.

**uvr B mutation**

The uvr B mutation is quite stable and can be confirmed by demonstrating uv sensitivity in strains that contain the mutation. For this, the cultures were streaked on the nutrient agar plate. The plate were partially covered (so that half of each streak was covered) with a piece of card board. The plates were then irradiated with a germicidal uv lamp at a distance of 33-35cm, for 8 seconds. The plates were then incubated overnight at 37°C after
which time it was observed that the bacteria grow only on the side that was not exposed to UV.

**R. Factor**

The presence of R. factor should be tested routinely by the presence of ampicillin resistance, because the plasmas are somewhat unstable and can be lost from bacteria (Mc Cann et al. 1975). For this, plate containing ampicillin (2.5JJ.g/ml) in the basal agar was prepared and the cultures were streaked on this. After 12.24 hours incubation at 37°C, growth was observed only along the streak made with the R. factor strains (TA 100 and TA 98).