MATERIAL AND METHOD
MATERIAL AND METHOD

The present study consisted of 30 cases of oral cavity lesion from the records in histopathological section of the Pathology department and also from the surgical wards of M.L.B. Medical College & Hospital, Jhansi were taken up to include benign and malignant lesions. One representative block of each case was chosen out.

All the cases were subjected to the following investigative methods.

1- Determination of blood groups in tissue and blood by
   (a) SRCA (Tonder & Kovarik et al, 1980)
   (b) Tile method (Dacie and Lewis, 1977) in fresh cases
   Different antisera were purchased from commercial sources.

2- Histopathological examination of tissues included
   i) Old H & E stained tissue sections from records were studied.
   ii) Wherever necessary sections were cut from fresh paraffin blocks and stained with H & E. In both the cases histopathological diagnosis and tumour typing was done.

3- SRCA (Tonder & Kovarik et al, 1980) was applied in all cases to assess the antigenic status.
SPECIFIC RED CELL AGGLUTINATION REACTION (SRCA):

PRINCIPLE:

The test is based on the three layered sandwich reaction, described by Davidsohn (1972) in which homologous bivalent or polyvalent antisera act as the connecting link between the isoantigen A, B or H present on the tissue as well as on indicator RBC (Fig No. 1).

In the three layered sandwich -
the bottom layer ---- is of tissue
the middle layer ---- is of homologous blood grouping antisera and
the top layer ---- is of homologous indicator red blood cells.

MATERIAL: The following material was used:

Tissue: Five micron thick sections from each block were mounted on separate microslides smeared with egg albumin. Dепaraffinization was done by passing the mounted section through xylene, 90%, 70% and 50% alcohol and water for short duration of time.

Antisera: Commercially available anti A, anti B with a titre of 512 and anti - H sera with a titre of 256 were used.
Indicator red blood cells: Blood samples belonging to group A, B and O were taken. RBC were washed in three changes of physiological saline and 1% suspension of RBC of each group was prepared in the same saline.

Physiological saline: 0.9 gm% sodium chloride solution in distilled water was prepared in the Chemical Laboratory of Pathology Department of I.I.E. Medical College, Jhansi.

PROCEDURE:

The test was performed in batches of 5-10 cases. Each slide was treated in the following manner:

1- The slide mounted with tissue section, was placed on a moist filter paper and antiserum was poured on the section and was covered with a petridish for 10 min at room temperature.

2- The uncombined antiserum remaining on the surface of the section was washed off in three changes of physiological saline each lasting for 10 minutes.

3- The excess saline was drained off and the individual slide was returned to the moist filter paper and covered with 1% suspension of indicator RBC for 10 min. at room temperature. Slide was covered with petridish in order to avoid drying.
4 - Another petridish was filled with minimal amount of physiological saline and the slide was turned upside down with a brisk movement and as such placed immediately on the two supporting wooden stick in the saline filled petridish so that it just touched the saline.

5 - After a few minute the slide was shifted over a clear area and after allowing 20-30 minute for indicator RBC that did not react specifically with the antiserum, to become detached and sink to the bottom of petridish, slide was finally moved aside on a clear area.

6 - The slide still remaining in the petridish was then examined with low power of microscope through the thickness of the slide with tissue section remaining on the lower surface using 5X and 10X eye pieces.

**CONTROL:**

To ensure that the SRCA reactions were specific the following controls were applied:

(A) **Tissue Control:** I - Intrinsic positive control -

1 - Endothelial lining of blood vessels
2 - RBC present in the section
3 - Epithelial cells of normal tissue adjacent to lesion

II - Intrinsic negative control - Connective tissue
(B) **Reagent Control**:

1- Heterologous antisera and homologous RBC were used e.g. In group A section Anti-B serum and group A RBC were used.

2- Homologous antisera and heterologous RBC were used e.g. in group A section Anti- A serum and group B RBC were used.

3- Blood grouping by SMCA reaction also served as a reagent control.

**INTERPRETATIONS**:

'-' Negative - No adhesion

'+ ' Doubtful positive - the result patchy with some areas show clear adhesion while other areas show no adhesion. Also included in this group are sections that show adhesion only in lower or top third of epithelium.

'+ ' Weak positive - all the cells do not show adhesion but weakly positive. Adhesion is diffuse not patchy.

'++' Moderately positive almost all cells show adhesion.

'+++ ' Strongly positive - over crowding of adhered red blood cells.