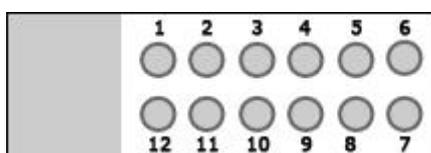




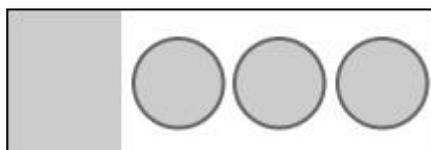
Application of Adhesion Slides

1) Principle

- a) In a new slide technique for investigation of cells, several areas are prepared to anchor cells. Viable cells can be permanently anchored to the glass surface without losing antigenicity or their ability to function.



- b) The standard type of the Adhesion Slide has two functional units:
- i) 3 or 12 reaction fields on whose glass surface the cells are anchored.
 - ii) The hydrophobic coating surrounding the reaction fields is very durable and effective in repelling even high concentrated protein solution. The hydrophobic coating prevents the solution from being mixed among the different reaction fields even if the slide is shaken on a vortex mixer.



- c) All kinds of cells can be tested:
- i) all blood cells such as lymphocytes, monocytes, granulocytes, thrombocytes and erythrocytes
 - ii) cells from bone marrow, effusions, liquor, bronchoalveolar lavage and cell suspension of lymph nodes and tumours



- d) Tests may be performed with only a few cells. In general, about 20'000 to 50'000 cells are used.
- e) The volume of substrate for incubation is small; 5 to 10 µl are sufficient to cover a spot.
- f) Washing of the cells is easily possible: performed by rinsing the spot with an adequate medium. Time consuming centrifugation is not necessary and cells loss can be avoided.
- g) The viable cells may be used and treated for many applications and studies. They may be fixed with different fixatives in aqueous solutions or they may be dried and fixed thereafter.

2) Preparation of the Adhesion Slide

- a) Spots of the Adhesion Slide are covered with a green stain to protect the Adhesion Coating. Covered with protection stain the Adhesion Slides can be stored at room temperature for couple of years.
- b) The protection stain of the Adhesion Slide is first to be dissolved under tap water, rinsed and then cleared of any residue with an isotonic buffer. An invisible film (Adhesion Coating) is left on the reaction fields whose positive charge makes for the electrostatic adhesion of the negatively charged cells.
- c) To prevent the Adhesion Coating drying out the processing is carried out in a wet chamber.
- d) Visible cell adhesion is ensured:
 - i) if the cells are viable, i.e. undamaged and
 - ii) if the washed cells are applied using a protein-free physiological buffer.
- e) Unsatisfactory adhesion and the loss of cells may be caused by following factors:
 - i) The green protection stain has not been removed entirely. Rinse completely with water and then rinse with isotonic buffer. Do not allow the adhesion coating to dry out.
 - ii) Mechanical damage to the adhesion coating. Damages by pipette tips or wiping are to be avoided.



- iii) Cell suspension has to be completely free of protein. The adhesion coating is neutralized by soluble proteins. Before application the cells are to be washed thoroughly in isotonic buffer without any added protein. Cells are to be applied immediately after isolation and washing.
- iv) Cells are damaged. Cell damage can be caused by long storage, non-physiological buffer or temperature shock by cold media. Damaged or dead cells adhere badly. When destroyed, they can give off substances which prevent the adhesion of other cells. Dead cells are to be removed.

The electrostatic adhesion of the cells on the slides is so stable that the reaction fields may be washed in the cuvette or carefully with a syringe without risking any cell loss.

3) Application of Adhesion Slide

- a) Immune peroxidase PAP test or comparable enzyme tests
- b) Immune fluorescence methods or other comparable methods
- c) Dyeing the cells using the Pappenheim method (morphology)
- d) Intra cellular antigen evidencing
- e) Molecular biologic methods, e.g. FISH



4) Selected Biography

Bross KJ, et. al.

Demonstration of cell surface antigens and their antibodies by the peroxidase-antiperoxidase method

Transplantation: 25: 331-334 (1978)

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A Hodgkin cell-specific antigen is expressed on a subset of auto- and alloactivated T (helper) Lymphoblasts

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Schneider H, et. al.

Identification of proliferating lymphocyte subpopulations in microcultures by surface marker and autoradiography

Immunological Communications
13: 553-561 (1984)

Frickhofen N, et. al.

Modified immunocytochemical slide technique for demonstrating surface antigens on viable cells

J of Clinical Pathology
38: 671-676 (1985)

Guzman J, et. al.

Tuberculous pleural effusions lymphocyte phenotypes in comparison with other lymphocyte-rich effusions

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5: 139-144 (1989)