“Good Quality Smear Examination makes A Good Quality TB Control Programme”

Akiko Fujiki
Contents

Introduction (2)

Chapter I: Preparation for Conducting the Course (3)
  Training Schedule (Suggested) (4)
  Major Material Requirements for Training in Stained Smear Preparation and Microscopy (5)
  Course Evaluation Sheet 1 (6)
  Course Evaluation Sheet 2 (7)
  List of Trainees by Their Institution and Date Attended (Sample) (8)

Chapter II: Training in Stained Smear Preparation (9)
  Flow Chart of Steps of Training in Stained Smear Preparation and Microscopy (11)
  Flow Chart of Training in Stained Smear Preparation (12)
  Assessment Points of Stained Smears (14)
  A Good Smear (16)
  Good and Poor Smears by Appearance (17)
  Form 1 (Worksheet for Smear Check) (18)
  Form 2 (Worksheet for Determining Proportion of Good Smear Preparation) (20)
  Form 3 (Feedback Sheet for Overall Smear Preparation by Radar Chart) (22)

Chapter III: Training in Microscopy (25)
  Flow Chart of Training in Microscopy (26)
  Code Numbers Table (28)
  Preparation of Slide Sets to Assign New Specimen Slide Numbers (29)
  Form A (Worksheet for Microscopy) (30)
  Form B (Worksheet for Microscopy for Consolidation of Results) (32)
  Form C (Feedback Sheet by Correlation Table) (34)
  Form D (Feedback Sheet by Scoring System) (36)
  Possible Causes of False Reading Results (38)

Chapter IV: Smear Microscopy Procedure (39)
  The Microscope and its Handling (40)
  Ziehl-Neelsen Staining Method (47)
  Microscopy of Acid Fast Bacilli (AFB) (52)
  Smear Scanning and Reporting Scale (53)
  Laboratory Request Form (54)
  NTP Laboratory Register (55)

References (56)
Appendix (57)
Introduction

“Keep it simple and practice a lot”

This is a training manual, which is expected to help trainers in conducting the practical training of Acid Fast Bacilli (AFB) microscopy. For this purpose, only the essential components of microscopy practice are described in the order of the training process and in the simplest and most practical manner. Also, this material will help trainers and trainees to identify problems or weaknesses in their practice and determine ways to overcome them.

As a result of the training, trainees should be able:
1) To demonstrate uniform skills on smearing, staining and smear microscopy.
2) To demonstrate the proper manipulation and maintenance of the microscope.
3) To properly record and report results in the National Tuberculosis Programme (NTP) tuberculosis (TB) laboratory registry.

This manual focuses on the more practical aspects of smear preparation and microscopy with a minimum of theory, as well as providing quick feedback on the skills from the trainers to the trainees during and after the training.

The details of AFB microscopy including training concepts are described in the booklet “Laboratory Services in Tuberculosis Control, Part I” by the World Health Organization (WHO) and “The Public Health Service National Tuberculosis Reference Laboratory and the National Laboratory Network” by the International Union Against Tuberculosis and Lung Disease (The Union).

Acknowledgments

Valuable input was received on producing this manual. Grateful acknowledgments go to Armand Van Deun (The Union), Shoichi Endo (RIT), Paula Fujiwara (The Union), Nobukatsu Ishikawa (RIT), Sang Jae Kim (The Union), Hans Rieder (The Union), and Geri Lennon (CDC) for reviewing the manuscript and providing invaluable suggestions and comments in the finalization of the draft.

Any part of this manual, including the illustrations, may be copied or adapted to local needs. We ask that you mention the name of the book as a source, using the following citation:

We would appreciate receiving a copy of any adapted materials or illustrations. Please send them to A.Fujiki, The Research Institute of Tuberculosis, 3-1-24 Matsuyama, Kiyose, Tokyo. E-mail: rit@jata.or.jp
Chapter I

Preparation for Conducting the Course

Training Schedule (Suggested)

Major Material Requirements for Training in Stained Smear Preparation and Microscopy

Course Evaluation Sheet 1

Course Evaluation Sheet 2

List of Trainees by Their Institution and Date Attended (Sample)

Proper planning and arrangements are important both before and during the training course to ensure smooth and cost-effective activities. This chapter introduces a simple management tool for conducting an effective training.
# Training Schedule (Suggested)

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Topics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>08:00 - 09:00</td>
<td>Registration</td>
</tr>
<tr>
<td></td>
<td>09:00 - 09:30</td>
<td>Opening ceremony / course orientation</td>
</tr>
<tr>
<td></td>
<td>09:30 - 12:00</td>
<td>(P) Microscope handling / Reagent preparation</td>
</tr>
<tr>
<td></td>
<td>13:30 - 17:00</td>
<td>(P) Initial smearing and staining</td>
</tr>
<tr>
<td>2</td>
<td>08:00 - 09:00</td>
<td>(L) AFB microscopy by Ziehl-Neelsen method</td>
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<tr>
<td></td>
<td>09:00 - 12:00</td>
<td>Recapitation, Questions and Answers</td>
</tr>
<tr>
<td></td>
<td>13:30 - 17:00</td>
<td>(P) Smearing, Staining, Reading</td>
</tr>
<tr>
<td>3</td>
<td>08:00 - 12:00</td>
<td>Recapitation, Questions and Answers</td>
</tr>
<tr>
<td></td>
<td>13:30 - 17:00</td>
<td>(P) Smearing, Staining, Reading</td>
</tr>
<tr>
<td>4</td>
<td>08:00 - 12:00</td>
<td>Recapitation, Questions and Answers</td>
</tr>
<tr>
<td></td>
<td>13:30 - 17:00</td>
<td>(P) Smearing, Staining, Reading</td>
</tr>
<tr>
<td>5</td>
<td>08:00 - 11:00</td>
<td>Overall technical evaluation</td>
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<tr>
<td></td>
<td>11:00 - 12:00</td>
<td>Course evaluation / Summary</td>
</tr>
<tr>
<td></td>
<td>13:30 - 14:30</td>
<td>Closing ceremony</td>
</tr>
<tr>
<td></td>
<td>14:30 - 17:00</td>
<td>Preparation for leaving</td>
</tr>
</tbody>
</table>

Topics and the time allocation mentioned above are only minimum requirements for this type of training. These may be modified according to the availability of time and knowledge and skill of the trainees; in particular, lecture sessions can be subject to change depending on local needs. The following lectures are minimal: 1) Safety precautions and disposal (30min.); 2) The DOTS strategy, including general information on the National TB programme (30min.); 3) Importance of AFB microscopy, including the function of the TB laboratory network (30min.); 4) Recording and reporting (30min.); 5) Ordering of laboratory supplies (30min.). Useful reference books for these topics are introduced on p. 56.
Major Material Requirements for Training in:

Stained Smear Preparation (quantity required for each trainee)

1) Glass slides (50 slides)
2) Stick/Wire loop for smearing (4 pieces of stick or 1 piece of wire loop)
3) Staining bridge (1)
4) Ziehl’s carbol fuchsin solution (200 ml)
5) 3% HCl ethanol or 20 - 25% H₂SO₄ solution (600 ml)
6) 0.3% Methylene blue solution (200 ml)
7) Microscope (1)
8) Immersion oil (5 ml)
9) Slide box for 100 slides (1 box)
10) Forms (Form 1, 4 sheets; Form 2, 1 sheet; Form 3, 2 sheets)
11) Fresh mucopurulent positive sputum specimen (1-2 ml)

Microscopy (for each trainee)

1) Panels of stained slides (1 set consists of 10 slides with known content of AFB)
2) Immersion oil (5 ml)
3) Microscope (1)
4) Xylene (to remove immersion oil from standard slides, 200 ml)
5) Forms (Form A, 1 sheet; Form B, 1 sheet; Form C, 3 sheets; Form D, 1 sheet)

Course Evaluation Sheet: Provide each trainee with a Course Evaluation Sheet 1 (see p. 6) at the end of the course. Collect all sheets and summarize their results on a single sheet (Course Evaluation Sheet 2). The latter sheet should be kept by the training manager for future reference to improve the course.

At least two (2) facilitators / instructors are required to conduct both training of stained smear preparation and microscopy.
Limit each group of trainees to no more than eight (8).
See the Appendix for a sample of the check list for the overall training arrangements. These may be modified according to the situation.
Course Evaluation Sheet 1
(to be filled out by each trainee)

1) Course:
   Duration : □ Just right □ Too short □ Too long
   Objectives achieved : □ Fully met □ Somewhat □ Not met
   Benefit obtained : □ Yes □ No □ Don’t know
   Training facilities : □ Good □ Fair □ Poor
   Tea break : □ Good □ Fair □ Poor
   Course coordination : □ Good □ Fair □ Poor
   Accommodation : □ Good □ Fair □ Poor
   Allowance : □ Just right □ Too much □ Insufficient

2) Sessions / Lectures:
   Time allocation : □ Just right □ Too short □ Too long
   Level : □ Just right □ Too high □ Too primary
   Subjects coverage : □ Just right □ Too broad □ Too specific

3) Reasons for answering “Fair/Poor” or “No / Don’t know”:

4) Topics which you think are not relevant to your job:

5) Topics about which you want to learn more:

6) Topics which were difficult to understand:

7) Other comments:
Course Evaluation Sheet 2
(to be used for summary report: number of participants are recorded)

1) Course:
   Duration : Just right ( )  Too short ( )  Too long ( )
   Objective achieved : Fully met ( )  Somewhat ( )  Not met ( )
   Benefit obtained : Yes ( )  No ( )  Don’t know ( )
   Training facilities : Good ( )  Fair ( )  Poor ( )
   Tea break : Good ( )  Fair ( )  Poor ( )
   Course coordination : Good ( )  Fair ( )  Poor ( )
   Accommodation : Good ( )  Fair ( )  Poor ( )
   Allowance : Just right ( )  Too much ( )  Insufficient ( )

2) Sessions / Lectures:
   Time allocation : Just right ( )  Too short ( )  Too long ( )
   Level : Just right ( )  Too high ( )  Too primary ( )
   Subjects coverage : Just right ( )  Too broad ( )  Too specific ( )

3) Reasons to answer “Fair / Poor” or “No / Don’t know”:

4) Topics which you think are not relevant to your job:

5) Topics about which you want to learn more:

6) Topics which were difficult to understand:

7) Other comments:
List of Trainees by Their Institution and Date Attended (Sample)

<table>
<thead>
<tr>
<th>No</th>
<th>Region or District</th>
<th>Institute</th>
<th>Name</th>
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<th>Cumulative Total</th>
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<td>Lab. K</td>
<td>Ms. A. Manons</td>
<td>&quot;</td>
<td>24</td>
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</tbody>
</table>

When the training manager plans a training program, he/she can identify which institution has or has not sent trainees through reviewing this list. This will help monitor whether all eligible technicians have been covered by the necessary training, and will avoid the repeated attendance of the same technician.
Chapter II

Training in Stained Smear Preparation

Flow Chart of Steps of Training in Stained Smear Preparation and Microscopy

Flow Chart of Training in Stained Smear Preparation

Assessment Points of Stained Smears

A Good Smear

Good and Poor Smears by Appearance

Form 1 (Worksheet for Smear Check)

Form 2 (Worksheet for Determining Proportion of Good Smear Preparation)

Form 3 (Feedback Sheet for Overall Smear Preparation by Radar Chart)

This chapter is designed to demonstrate the operational steps using appropriate forms. Explain the outline of the training steps before starting practice (pp. 11-13).

Assess the skills of each trainee using the forms to identify what improvements should be made at each practice. Throughout the practice sessions, the trainee can learn his/her own skill status by quick feedback from and discussions with the trainer.

The trainee also learns how to assess the technical skills by using the forms.
The main objective of the practice given to the trainees is to standardize and to enhance their skills on AFB microscopy.

Chapters II and III are designed to demonstrate the operational steps using appropriate forms.

A flow chart of the steps to cover both stained smear preparation and microscopy is shown on page 11.

Learning Through Seeing, Doing, And Thinking

An adaptation of “Helping Health Workers Learn by D. Werner and B. Bower”
Flow Chart of Steps of Training in:

**Stained Smear Preparation**

I. Practice before Training (initial practice)
   - **Trainee**
   - Forms 1, 2, 3

II. Lecture / Instruction
   - **Trainer**

III. Practice after lecture / instruction
   - **Trainee**
   - Forms 1, 2, 3

IV. Evaluation after practical training
   - **Trainer**
   - Forms 2, 3

V. Feedback
   - **Trainer**

**Microscopy**

I. Preparation of panels of stained smear slides
   - **Trainer**
   - Rearrange slides by code numbers table

II. Reading of slides
   - **Trainee**
   - Form A

III. Analysis of results
   - **Trainer**
   - Forms A, B, C, D

IV. Feedback
   - **Trainer**

---

*Good Quality Smear Examination makes A Good Quality TB Control Programme*
Flow Chart of Training in:

**Stained Smear Preparation**

I. Practice before Training (initial practice)
   - Trainee
   - Forms 1, 2, 3

II. Lecture / Instruction
   - Trainer

III. Practice after lecture / instruction
   - Trainee
   - Forms 1, 2, 3

IV. Evaluation after practical training
   - Trainer
   - Forms 2, 3

V. Feedback
   - Trainer

---

**Good Quality Smear Examination**

makes

**A Good Quality TB Control Programme**
Training in Stained Smear Preparation

I. Practice to evaluate the proficiency of trainees before training (initial practice)
   1) Ask trainees to prepare 10 slides of sputum smears and stain them by the Ziehl-Neelsen method.
   2) Do not instruct trainees on how to do smear preparation or training, in order to be able to discover the weak points of the trainees.
   3) Collect all prepared slides.
   4) Evaluate these slides by using the six checkpoints (see pp.14-15). The score obtained is the baseline value for comparison with post training results. The evaluation sheet is based on Form 1 and Form 2. The results of the six overall checkpoints are expressed by using Form 3.
   5) For purposes of confirmation, keep all slides until the end of the training.

II. Lecture / instruction on smear examination by the Ziehl-Neelsen method
   1) Focus primarily on technical points during instruction of smear preparation and staining by the Ziehl-Neelsen method (see Chapter IV).
   2) Emphasize evaluation of weak points that were found during the initial practice.
   3) Instruct trainees how to rectify and correct these weak points.

III. Practice after lecture / instruction
   1) Request trainees to repeat the practice on preparation of smears and staining. Each trainee should prepare 10 sputum stained smears.
   2) Evaluate the slides by using Form 1, Form 2, and Form 3, in the same manner as the initial practice session.
   3) Repeat this practice as many times as necessary until each trainee obtains an overall score greater than 90%. Keep the slides chronologically until the end of the training, as they serve as feedback for discussion.

IV. Evaluation after practice training
   1) Evaluate progress of the stained smear preparation by observing the actual stained smears together with the overall evaluation score (Form 3).
   2) Evaluate each trainee individually on whether his/her proficiency has reached a satisfactory level (Form 2, Form 3). The results of each practice may be used for evaluation.
Assessment Points of Stained Smears

Based on these criteria, calculate the proportion (%) of good quality of each check point and plot the score of each check point on Form 3 to observe overall results (see pp. 22-23).

**Sputum Quality Check** (by microscopic observation)

1) **The presence of leucocytes**
   The presence of more than 25 leucocytes per field (at 100 × magnification) or presence of dust cells (macrophages) indicates good quality sputum.

![Leucocyte (×100)](image1) ![Dust cell (×1,000)](image2)

**Smear Preparation Check** (by macroscopic and microscopic observation)

2) **Smear area size**
   An acceptable size of a smear should be approximately 1-2 cm × 2-3 cm. One (1) horizontal line in a 1×2 cm smear or in a 2×3 cm smear would cover about 100 to 150 microscopic fields.

![Size of 2×3 cm](image3) ![Size of 1×2 cm](image4)

3) **Evenness of smear**
   Sputum should be spread evenly on the glass slide: not too thick and not too thin.

![Good](image5) ![Good](image6) ![Sloughed off](image7) ![Uneven](image8)
4) Thickness of smear

A smear of acceptable thickness before staining can be checked by looking at printed letters through the smear, holding the smeared glass slide 4-5 cm over a piece of printed paper before staining (see p. 16). If the letters cannot be read, it is too thick. A stained slide can be checked for appropriate thickness by observing it under the microscope; if the entire depth of the smear layer can be focused sharply in each field, its thickness is acceptable.

![Good, Too thick, Too thin images]

**Staining Techniques Check** (by microscopic observation)

5) Decolourizing condition by Ziehl-Neelsen stain

Ziehl's carbol fuchsin in the smear should be removed by applying a decolourizing agent such as acid-alcohol or sulphuric acid. If Ziehl's carbol fuchsin stain is retained in the stained smear, it must be evaluated as poorly decolourized.

![Good, Under-decolourization images]

6) Smear cleanliness

The stained smear must be free from stain deposits, dirt, debris, crystals produced by overheating during staining.

![Good, Dirt with crystals images]
A Good Smear

A smear of acceptable thickness before staining can be checked by looking at printed letters through the smear, holding the smeared glass slide 4-5 cm over a piece of printed paper before staining. If the letters can not be read, it is too thick.

A Good Smear is:

Made from mucopurulent sputum
Spread evenly using a repeated coil type form
Approximately 2-3 cm × 1-2 cm in size
Not too thick
Thin enough to read through newsprint after drying but before staining
Air dried before being fixed by flaming to prevent slough off

stop TB
Good and Poor Smears by Appearance

**Good Smear**  
- Too thick
- Too big
- Uneven
- Too big
- Under-decolourization
- Straight line smear
- Uneven
- Too small
- Uneven
- Sloughed off
- Sloughed off
- Too thin
- Uneven
- Uneven
- Too thick
- Sloughed off
- Under-decolourization

**Poor Smear**
# Form 1  Worksheet for Smear Check

Date: ______________ No. of Smears Prepared: ____ Name of Trainee: ____________

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Specimen Quality</th>
<th>Staining</th>
<th>Cleanness</th>
<th>Thickness</th>
<th>Size</th>
<th>Evenness</th>
<th>Remarks</th>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

*K* = Too thick  *N* = Too thin  *S* = Too small  *B* = Too big

Remarks:

Assessor: ___________________________
A quality smear can be evaluated in terms of the six checkpoints. The assessment points and their criteria are described on pp. 14 and 15.
Form 2 Worksheet for Determining Proportion of Good Smear Preparation

Name of Trainee: ______________________

<table>
<thead>
<tr>
<th>Date</th>
<th>Check point</th>
<th>Before (initial)</th>
<th>After 1</th>
<th>After 2</th>
<th>After 3</th>
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<td>Staining</td>
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<td>Cleanliness</td>
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<td>%</td>
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</tr>
</tbody>
</table>

G = Good quality

Before (initial) = Initial practice; Results before being given instruction on smear techniques.
After 1-3 = Results after having received proper instruction on smear techniques.
### Form 2 Worksheet for Determining Proportion of Good Smear Preparation

Name of Trainee: **A. Abcde**

<table>
<thead>
<tr>
<th>Date</th>
<th>Check point</th>
<th>Before (initial)</th>
<th>After 1</th>
<th>After 2</th>
<th>After 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Slides</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specimen Quality</th>
<th>G</th>
<th>0</th>
<th>10</th>
<th>10</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<table>
<thead>
<tr>
<th>Staining</th>
<th>G</th>
<th>2</th>
<th>10</th>
<th>10</th>
<th>10</th>
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<tbody>
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<td>100</td>
<td>100</td>
<td>100</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Cleanliness</th>
<th>G</th>
<th>0</th>
<th>8</th>
<th>10</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>0</td>
<td>80</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Thickness</th>
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<th>0</th>
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<th>10</th>
<th>9</th>
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<tbody>
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<td>%</td>
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<td>90</td>
<td>100</td>
<td>90</td>
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</table>

<table>
<thead>
<tr>
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<th>8</th>
<th>10</th>
<th>10</th>
</tr>
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<tbody>
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<td>80</td>
<td>100</td>
<td>100</td>
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</table>

<table>
<thead>
<tr>
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<th>G</th>
<th>0</th>
<th>6</th>
<th>9</th>
<th>9</th>
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<tr>
<td>%</td>
<td>0</td>
<td>60</td>
<td>90</td>
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</tr>
</tbody>
</table>

**G** = Good quality

Before (initial) = Initial practice; Results before being given instruction on smear techniques.

After 1-3 = Results after having received proper instruction on smear techniques.

Proportion (%) of smears of good (G) quality of each checkpoint is calculated

\[
\left( \frac{\text{Number of good quality of each checkpoint}}{\text{Total slides prepared}} \right) \times 100
\]

and scores of each checkpoint are plotted on **Form 3** (see pp. 22-23).
Form 3  Feedback Sheet for Overall Smear Preparation by Radar Chart

Name of Trainee: ________________

Specimen Quality (%)

(Date of practice: ____________)

Clearness(%)  Staining(%)

Thickness(%)  Size (%)

Evenness(%)
Form 3  Feedback Sheet for Overall Smear Preparation by Radar Chart

Name of Trainee: A. Abcde

(Date of practice: 17 April 2004 Before/Initial)

(Date of practice: 18 April 2004 After 1)

(Date of practice: 19 April 2004 After 2)

(Date of practice: 20 April 2004 After 3)