Oesophageal Squamous Cell Carcinoma African Prevention Effort

ESCCAPE-Tanzania

CASE-CONTROL STUDY MANUAL
Full study 2015-2017

Version 1 October 2015

Local ESCCAPE contact telephone: 0683 105298

International Agency for Research on Cancer

World Health Organization

Abbreviations and Key Definitions, alphabetical Order

APW	Agreement for the Performance of Work (type of contract with IARC)
Case	Patient newly diagnosed with confirmed or suspected oesophageal cancer
Control	Hospital patient or hospital visitor who does not have ANY type of cancer, either now or in the past, i.e. cancer-free individual
Dx	Diagnosis
EC	Esophageal cancer (all histologies)
ESCC	Esophageal Squamous Cell Carcinoma (the histology type of primary interest)
ESCCAPE	Esophageal Squamous Cell Carcinoma African Prevention Effort
H&S	Health and Safety
IARC	International Agency for Research on Cancer
IEC	IARC Ethics Committee
KCMC	Kilimanjaro Christian Medical Centre
KCRI	Kilimanjaro Clinical Research Institute
NA	Not applicable
NK	Not known
PI	Principal Investigator
SI	Study interviewer

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IDs

110XXX	Case or Control being interviewed at KCMC
120XXX	Case being interviewed at Machame hospital, Siha DH or Huruma Hospital

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Contents

1. ESCCAPE overview

The Oesophageal Squamous Cell Carcinoma African Prevention Effort (ESCCAPE) is a collaborative research effort being conducted across African countries, co-ordinated by WHO's specialized cancer agency, the International Agency for Research on Cancer (IARC) and in collaboration with international partners in the UK and US. ESCCAPE aims to investigate the aetiologic epidemiology of squamous cell carcinoma in Africa. This cancer has a most intriguing geographic epidemiology worldwide. Focal areas of high incidence exist in Asia – the central Asian belt stretches from Iran to parts of China, in the Eastern Cape Province of South Africa, and parts of Brazil and Uruguay. In Africa the spatial variations in incidence are also immense. This cancer ranks amongst the top three cancers in several East African countries and eastern parts of Southern Africa, but intriguingly it is extremely rare in West Africa. Its prognosis is poor, thus if modifiable factors are responsible for the disproportionately high oesophageal cancer incidence rates in East Africa, identifying them will inform the most effective primary prevention efforts.

In Africa, the main histological type of oesophageal cancer (EC) is oesophageal squamous cell carcinoma (ESCC). Typically patients are diagnosed at late stages, and most die within months. No large-scale studies have been conducted to systematically investigate the potential causes of this cancer in East Africa. ESCCAPE aims to fill this research-evidence gap.

ESCCAPE encompasses a series of parallel activities that aim to shed light on the causes of oesophageal cancer in the African EC corridor. The activities include:

- Cross-sectional studies on the prevalence of risk factors that have been established as ESCC risk factors in other settings of the world
- Ecological studies linking population-level risk factors to population-level EC incidence rates
- Case-control studies of oesophageal cancer. In sites where a case-control study can be undertaken, at a minimum a 'core' study protocol is being followed which includes the recruitment and interviewing of newly diagnosed oesophageal cancer patients (CASES) and a comparison group of controls. The only biospecimens taken in this core protocol are saliva samples. In the full-study protocol, i.e. where endoscopy and freezers are available, we are additionally collecting a blood sample and urine from all participants and tumour tissue from cases.

ESCCAPE's ***research strategy*** is based on the following broad perspectives. Firstly, a broad viewpoint on putative risk factors has been adopted. We are studying the individual and combined effects of a broad range of lifestyle, infectious, environmental and genetic risk and protective factors. Secondly, descriptive and analytical studies are being undertaken in multiple African settings, with a view to interpret findings holistically in terms of their

relevance, if at all, to the peculiar distribution of the entire African oesophageal cancer corridor. Thirdly, we consider that the priority factors that need investigating are those that have already been established as carcinogenic to humans, or probably carcinogenic for squamous cell oesophageal cancer in non-African settings. Such factors are being evaluated with respect to their population-level exposure levels and local sources as well as their contribution to the cancer burden.

The factors being studied as **causal factors or risk markers** include:

- socioeconomic indicators
- occupation
- alcohol consumption
- smoked and inhaled products
- polycyclic aromatic hydrocarbons (PAH) exposures
- hot (by temperature) foods and drinks
- fruit and vegetable intake
- pickled and salted vegetables
- tooth loss
- micronutrient deficiencies
- nitrosamines
- infections
- mycotoxins
- genetic susceptibility.

Most of the factors being studied are modifiable, thus if they are found to be causes of oesophageal cancer in the African EC corridor, interventions could be implemented to remove exposures to carcinogens (e.g. advice on alcohol intake, particularly stronger spirits), or through bio-fortification means.

mHealth! Where possible, we are implementing all data collection using mobile phones or tablets for **real-time data collection** via tailored applications, or on tablets. This serves to ensure efficient standardized and higher-quality data collection through immediate quality control implementation. A close connection is attained on progress and interactions are attained across ESCCAPE collaborators, whilst maintaining confidentiality of personal identifying participant information.

The **ESCCAPE consortium** is coordinated by IARC's Section of Environment and Radiation, Dr Valerie McCormack, to facilitate collaboration and standardization of methodology across centres during study preparation, fieldwork and analyses and reporting. ESCCAPE study sites to date include focal points at:

- **Kenya**: Moi Teaching and Referral Hospital, Eldoret, West Kenya
- **Tanzania:** Kilimanjaro Christian Medical Centre/Kilimanjaro Clinical Research Institute, Moshi, North Tanzania

Plans to expand to other East African countries, especially to Malawi and/or Uganda, are being developed. Through the collaborative work, the combined expected recruitment in Eldoret (150 cases/year) and Moshi (100 cases/year), we could accrue a case control study with 750 cases over 3-4 years, creating a powerful informative resource for EC in Africa.

ESCCAPE-Tanzania encompasses several activities in close collaboration with KCRI and KCMC aimed at investigating the excess burden of EC in the Kilimanjaro Region of North Tanzania. To date ESCCAPE-Tanzania has already conducted several activities which formed important groundwork activities:

- a cross-sectional study in the Machame community to investigate the prevalence of risk factors for this cancer that have been observed in other areas of the world; including of measured tea temperatures
- analysis of the cancer registry data and mapping of EC cases in the region.

The present study manual is for the launch of the pilot fieldwork phase for a case-control study of EC in the Kilimanjaro region.

2. Collaboration details

2.1 Website

A brief website for ESCCAPE has been created. This should serve to publicize the consortium to other potentially interested investigators, new sites and postdoctoral fellows. The website is hosted by IARC at http://esccape.iarc.fr.

2.2 Investigators

ESCCAPE Tanzania	Kilimanjaro Christian Medical Centre / Kilimanjaro Clinical Research Institute, Moshi, Tanzania: Dr Prof Blandina Mmbaga Dr Amos Mwasamwaja Dr Michael Oresto Munishi - currently commencing a PhD in Tampere, Finland, which will be based on this present work Dr Gibson Kibiki
ESCCAPE IARC Team	PI Dr Valerie McCormack (epidemiology, statistics) Dr Joachim Schüz (epidemiology, bioinformatics) Dr Behnoush Abedi-Ardekani (pathology)
ESCCAPE-Eldoret	PI Dr Diana Menya

2.3 Funding

- ESCCAPE Eldoret pilot case-control study was funded by IARC.
- ESCCAPE Eldoret case-control full study is supported by a US National Cancer Institute Grant R21 CA191965-01, commencing 30th January 2015, for a duration of 2 years.
- ESCCAPE Tanzania pilot study (October 2015) for minimum 12 months is funded by IARC. External funds are being sought for continuation of the pilot study.
- Dr Oresto-Munishi (KCMC cancer registry) received a UICC-IARC fellowship in 2014 to work in this area.

3. ESCCAPE-Tanzania pilot case-control study

3.1 Overview

A case-control study of oesophageal cancer will be piloted in the Kilimanjaro region. In this study design, we compare exposure histories in CASES - who are patients with confirmed or suspected oesophageal cancer - to exposure histories in CONTROLS. The latter are a comparison group that represent the communities/populations where the cases originate.

In sites where a case-control study can be undertaken, at a minimum a 'core' study protocol is being followed which includes the recruitment of newly diagnosed oesophageal cancer patients (CASES) and a comparison group of controls. Controls are frequency matched to the age and sex distribution of cases. Each consenting case and control is asked to complete a face-to-face interview on risk factors using a structured questionnaire. The only biospecimen taken in this core protocol is a saliva sample. In the full-study protocol, i.e. where endoscopy and freezers are available, we will ask participants to provide blood and urine and, for cases, tumour tissue.

Table 3.1: Overview of recruitment sites and recruitment groups

Site	**Protocol**	**Cases** **(patients with confirmed/suspected esophageal cancer**	**Controls** **(hospital patients or visitors without esophageal cancer)**
KCMC	*Full*	***GROUP 1*** Endoscopy Study interview Saliva sample Blood Urine Tumor biopsy	***GROUP 4*** Study interview Saliva sample Blood Urine
Machame Hospial Siha DH Huruma	*Core*	***GROUP 2*** Presentation details Study interview Saliva sample Provide study card. Refer to KCMC	None
Kilema, St.James,	*Referral only*	***GROUP 3*** Provide study card. Refer to KCMC	None

3.2 Aims and objectives

Pilot Study

Aim: To set up a case-control study of esophageal cancer in the Kilimanjaro region
Objectives:

a. Identify recruitment sites for cases of squamous cell esophageal cancer in the Kilimanjaro Region
b. Evaluate the case patient profile and biospecimens collections possible at each recruitment site
c. Examine study participation rates in cases and controls, by study recruitment sites, to the various parts of the study (questionnaire, various biospecimens)
d. Train study research assistants in the conduct of all study procedures
e. Tweak the ESCCAPE mHealth application for effective use by fieldworkers
f. Recruit controls at KCMC and compare their residential origins to that of the cases
g. Inform changes needed to implement a successful full case-control study

Longer-term aims

- To investigate the relation of ESCC risk factors established in other settings to ESCC risk in Tanzania
- To express ESCC risks in terms of local sources of exposures, to estimate their contribution to the disease burden in the African EC corridor
- To pool data from all ESCCAPE sites, so as to increase power for the assessment of ESCC risk factors in Africa. To compare the contributing risk factors across ESCCAPE settings.
- To commence a bio-banking resource for EC in Arica, to enable investigations of more refined mutation spectrum and exposure-specific epigenetic signatures.

3.3 Study setting

This site is in Tanzania, East Africa, bordering Kenya to the north, Uganda, Rwanda, Burundi and Lake Victoria on the north-west, to the south Zambia, Malawi and Mozambique and its east coast is on the Indian Ocean. Kilimanjaro Region is the focus of the study – this is one of the 19 administrative regions in Tanzania, as shown below, with its main relief feature of the Kilimanjaro Mountain that rises to 5895m in the north of the region, between the Siha (a new district, not in map), Hai, Rombo and Moshi rural districts. Amongst the 1.6 million population of the region, demographic features of interest include a female excess (845,000 women and 795,000 men) and a young population: 37.8% aged 0-14 years, 55.1% aged 15-64 and 7.0% aged 65 years and older (Tanzanian 2012 census data). This proportion of the population that is elderly is, however, the highest proportion of all Tanzanian Regions. In the region, 1.2 million are rural residents, 0.4 million urban.

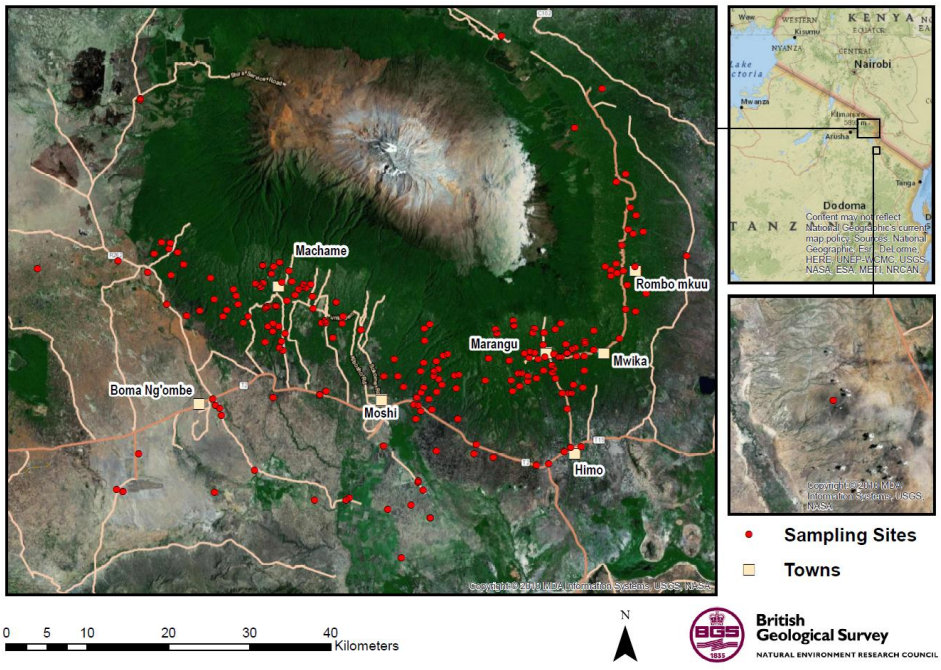
District of Kilimanjaro Region	Population (Number)	Number of Households	Average Household	Sex Ratio Men per

			Size	100 women
Both Sexes				
Rombo District Council	260,963	59,871	4.4	91
Mwanga District Council	131,442	30,197	4.4	93
Same District Council	269,807	59,957	4.5	95
Moshi District Council	466,737	110,806	4.2	94
Hai District Council	210,533	50,648	4.2	95
Moshi Municipal Council	184,292	46,169	4	94
Siha District Council	116,313	27,205	4.3	94
Kilimanjaro Region	1,640,087	384,853	4.3	94

Tanzania, showing Kilimanjaro region

Districts of Kilimanjaro

Geo-mapping of the approximate residential location of ESCC patients diagnosed at KCMC but not residing in Moshi itself during 2005-10 is shown below.



4. Approvals

4.1 Ethics approval

The study has been approved both in France (IARC ethics committee, IEC) and Tanzania. The IEC requested that in the instance of an oesophageal cancer patient being diagnosed under age 18, they should NOT be recruited to the study. This is reflected in the eligibility criteria for cases. Accordingly, NO minors below the age of 18 years should be invited as controls. The Tanzania approval was granted under KCRI PI Prof. Kibiki and we are in the process of updating the Dr Amos Mwasamwaja.

International Agency for Research on Cancer

→ For Val

World Health Organization

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http://ethics.iarc.fr/

Dr Valerie McCormack
Section of Environment and Radiation (ENV)
IARC

Ref.: IEC 14-15

6 August 2014

Dear Dr McCormack,

You submitted further information concerning the following project for review by the IARC Ethics Committee (IEC):

Project No. 14-15

Esophageal Squamous Cell Carcinoma in Africa Prevention Effort (ESCCAPE)

This project was evaluated by the IEC during its meeting on 18 June 2014. The members present were as follows:

M. Baduraux (external member)
E. Bayle (IARC)
B. Fervers (IEC Chair, external member)
G. Scélo (IARC)
E. Seleiro (IARC)
H. Storm (external member)
S. Vaccarella (IARC)
P. Vineis (IEC Vice-Chair, external member)

The IEC would like to thank you for your thorough responses to the points that required clarification, in your cover note dated 4 June 2014.

The decision of the IEC concerning the above project is as follows:

Approved after ethical review. No annual report required.

Yours sincerely,

Dr B. Fervers
Chair, IARC Ethics Committee

THE UNITED REPUBLIC OF
TANZANIA

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27th July 2015

Prof Gibson Kibiki
Kilimanjaro Clinical Research Institute (KCRI)
P O Box 2236, MOSHI, Kilimanjaro.

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Esophageal Squamous Cell Carcinoma African Preventive Effort (ESCAPE, (Kibiki G *et al*), has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: KCMC Endoscopy Unit, and in Kilimanjaro region.

Approval is for one year: 27th July 2015 to 26th July 2016.

Name: Dr Julius J Massaga

Signature
Ag CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

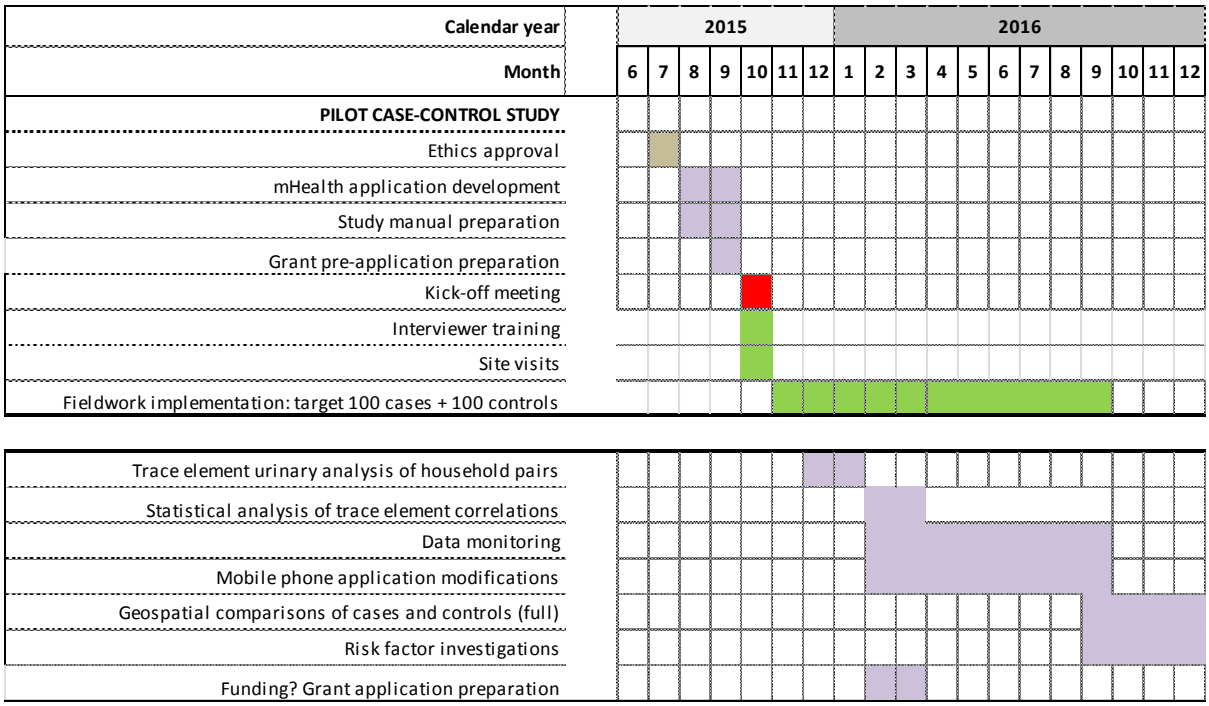
Name: Dr Margaret E Mhando

Signature
Ag CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL WELFARE

CC: **RMO**
DED
DMO

5. Study timeline

ESCCAPE-Moshi received seed funding in September 2015 from IARC. A kick off meeting (in red below) will bring together all those involved, and will be held on 21st October 2015. The work plans during this first year, including plans to attract funding to prolong research activities are time-tabled below.



6. Site and equipment requirements: Pre-fieldwork set-up

6.1 Interview area

This interview space needs to:

- be a private area for the conduct of study interviews
- be situated near the wards or upper GI endoscopy clinic where new EC patients are seen. Patients should be able to reach this office/space easily, e.g. not by climbing up many flights of stairs. At the study interviwer's (SI) discretion and subject to the patient's comfort and agreement, interviews can also take place at the patient's bedside
- have space for lockable storage of the phone/tablet and of study materials and equipment
- have an electricity supply to recharge mobile phones/tablets

6.2 Study materials

Y=YES N=No.	KCMC	Machame, Siha, Huruma Hospitals	St. Joseph's Mawenzi Kibosho Kileman Marangu
6.2.1. **Equipment**			
Weighing scales	Y	Y	N
Stadiometer (fixed or mobile)	Y	Y	N
Mobile phone/tablet for questionnaire data (Android OS)	Y	Y	N
Lockable filing cabinet for all paper study materials	Y	N	N
Tumour cabinet (IARC to provide)	Y	N	N
Tumour slide box (IARC to provide)	Y	N	N
6.2.2. Phone contracts			
Insurance for the mobile phone	Y	Y	N
Small data plan for phone	Y	Y	N
6.2.3. Consumables			
Ink pad (for fingerprint consent)	Y	Y	N
In study folder/with Study interviewer			
Log file of eligible patients.	Y	Y	N
Consent forms	Y	Y	N
Participant information sheets, for cases+ controls	Y	Y	Y
Paper-based backup questionnaires	Y	Y	N
Barcodes (provided by IARC)	Y	Y	N
Study cards for patients (provided by IARC)	N	Y	Y
At endoscopy unit/presenting location			
Oragen DNA Saliva kits (provided by IARC)	Y	Y	N
RNA later tubes (provided by IARC)	Y	N	N
Consumables in laboratories			
5 ml EDTA vacutainers	Y	N	N
5 ml plain vacutainers	Y	N	N
1 ml cryovials	Y	N	N
5 ml cryovials	Y	N	N
Urine collection containers 50ml	Y	N	N

6.2.1. **Equipment**

Weighing scales
Stadiometer (fixed or mobile)
Mobile phone/tablet for questionnaire data (Android operating system)
Lockable filing cabinet for all paper study materials, consent forms, phones/tablets
Tumour cabinet (IARC to provide)

Tumour slide box (IARC to provide)

6.2.2. Phone contracts

Insurance for the mobile phone
Small data plan for phone

6.2.3. Consumables

Ink pad (for fingerprint consent)

In study folder/with Study interviewer

Log file of eligible patients
Consent forms
Participant information sheets, separately for cases and controls
Paper-based backup questionnaires
Barcodes (provided by IARC)
Study cards for patients (provided by IARC)
Urine collection containers 50ml

At endoscopy unit/presenting location

Oragen DNA Saliva kits (provided by IARC)
RNA later tubes (provided by IARC)

Consumables in laboratories

5 ml EDTA vacutainers
5 ml plain vacutainers
1 ml cryovials
5 ml cryovials
Urine collection containers 50ml

Mobile phones

Recommended phone/tablet for using the Mobenzi application is the Samsung Galaxy Tab. Each study interviewer will be responsible for the safe-keeping of their assigned study phone. Study phones are to be used for study purposes only. The local PI should liaise with the IARC before purchasing a phone.

All equipment should be securely stored when not in use.

7. Study interviewer: Suitable person and their responsibilities

A competent responsible study interviewer(s) (SI) is key to this study, as they have all contact with patients and will overview the full process to obtain information and biospecimens for participants, and ensure their appropriate storage.

7.1 Study interviewer recruitment

Study Interviewers need to be highly motivated and driven, and have a commitment to high quality data collection and organisation. The desirable qualities of the SI are: (i) have excellent personal skills and a caring manner for the appropriate repeated contacts with patients; (ii) familiarity with the oncology/endoscopy clinic and its patient records; (iii) competence with the use of smart Android mobile phones, internet access, use of email and computers; (v) willingness to learn a mobile-phone based data entry system; (vi) if possible, to take blood from patients, and if not, to make arrangements therefor.

The KCMC SI should be dedicated to working for the ESCCAPE case-control study and have the flexibility to be available for participant interviews within the time period that the case is at the hospital (sometimes only one day). SIs are required to keep in regular contact with the local PI and with IARC throughout the study fieldwork.

Conducting interviews: The study interviewers have a key role in obtaining information from study participants and they need to so in a manner so as not to influence the participant's answers in any way, neither for the cases or controls. A consistent approach to asking questions is to be maintained throughout all interviews; such consistency is aided by proceeding with the interview from start to finish without interruption. All information recorded in the interview responses should have been provided by the subject, with no information assumed by the interviewer. So as not to influence a participant's later responses, the interviewer must remain neutral and objective throughout the interviewer. In particular the interviewer should accept all responses and make sure not to react to any of the responses provided (e.g. reacting surprised, disappointed, pleased to particular responses) as this is likely to affect subsequent responses. Maintain such a neutral stance can be achieve whilst still remaining interested when conducting the interview.

7.2 Study interviewer training, support and supervision

Each SI will receive training on study procedures, and be provided with study manuals for constant reference. When appropriate, the SI will also receive training on the Mobenzi mobile phone application for data capture and study management.

7.3 Study interviewer responsibilities

The SI will be expected to:

- Ensure fieldwork documentation and equipment are sufficient and working.

- Interview all newly oesophageal cancer patients who consent to participate in the study
- Extract clinical and pathology information from the case patient's clinical records
- Arrange for a back-up for absences
- Be responsible for the mobile/tablet handset. This is an expensive item and should not be left unaccompanied at any time. Please do not leave it unaccompanied even for a few minutes.

In conducting fieldwork, the SI is expected to:

- Maintain a high quality of study conduct throughout.
- Maintain contact with the local PI at least every two weeks, with a fieldwork update
- Alert either the local PI or IARC if any circumstances arise that may affect the study conduct. No question is irrelevant or too small. All questions asked to the ESCCAPE team at an individual site will be helpful for the overall study conduct across all sites, as problems may repeat in other centres, and clarifications can be incorporated into subsequent versions of this study manual.
- Inform the local PI and IARC of circumstances that impinge on study quality or participant recruitment. Examples include:
    - If fieldwork is suspended for any period of time. Please include information on the reason for suspension, and specify the dates and times fieldwork was suspended or postponed.
    - Patients are having difficulties in certain parts of the questionnaire or in bio-specimen collections.
    - In advance, the SI should advise the local PI when his/her leave is scheduled, and fieldwork arrangements and back-up plans in place for the period of leave.

The SI should act professional at all times; while still ensuring a good rapport with the participants. Always keep in mind that this is a very difficult time for the participants, especially the case patients newly diagnosed, and we do not want to cause any additional stress.

Three fundamentals of all patient contact must be kept in mind at all times:

- Informed **consent** must be obtained
- All patient details are to be kept **confidential**
- Patients are **free** to participate or withdraw as and when they want without their care being affected in any way.

7.4 Study Interviewer: List of Once-off and Repeated Tasks

3 monthly Tasks for the KCMC study interviewer/coordinator only

Visit all the peripheral sites in Siha, Rombo and Machame. Transfer the consent forms and collected saliva kits to KCRI for storage with the remaining study materials.

Monthly Tasks

1. Make photocopies of:
    a. Participant information sheet (in study folder)
    b. Consent form (in study folder)
    c. Back-up questionnaires (in study folder)
    d. Presentation form (in endoscopy unit/ENT/all places where patients present)
    e. Pathology form
    f. Check with local PI/IARC on the age-sex distribution for cases and controls*

**It is always a priority not to miss an eligible case. Controls are recruited from a larger potential number of people, however we should not fall behind on their recruitment otherwise we must introduce differences due to different months or seasons of recruitment*

Weekly Tasks

2. Double-check there are sufficient documents in 1.
3. *If using Mobenzi,* click on "check for updates". This will download any changes that have been made to the questionnaires.

Daily Tasks

5. Recharge mobile phone/tablet.
6. Enter new eligible participants into log file.
7. Enrol cases and controls.

7.5 Study interviewer back-up

Provisions should be in place for days when the SI is unexpectedly absent or delayed. In these instances basic contact information (name, telephone number) should be collected from newly presenting oesophageal cancer cases, so the SI can trace the patient to complete the full interview. In circumstances where no back up person is able to interview, a log book in which the names and contact details of all eligible patients are routinely recorded should be used. The person identified as the back-up interviewer should be briefed about the study and shown the study materials folder.

8. Overview: Participant eligibility, data and specimen collection process

A case-control study of oesophageal cancer will be piloted in the Kilimanjaro region. In this study design, we compare exposure histories in CASES who are patients with newly diagnosed oesophageal cancer to exposure histories in CONTROLS. The latter are a comparison group that represent the population. We will analyse what factors differ between cases and controls, and for those that do, whether they are likely to be reasons that contribute to a greater likelihood of ESCC.

Table 3.1 (repeated): Overview of recruitment sites and recruitment groups

Site	**Protocol**	**Cases** **(patients with confirmed/suspected esophageal cancer**	**Controls** **(hospital patients or visitors without esophageal cancer)**
KCMC	*Full*	***GROUP 1*** Endoscopy Study interview Saliva sample Blood Urine Tumor biopsy	***GROUP 4*** Study interview Saliva sample Blood Urine
Machame Hospial Siha DH Huruma	*Core*	***GROUP 2*** Presentation details Study interview Saliva sample Provide study card. Refer to KCMC	None
Kilema, St.James,	*Referral only*	***GROUP 3*** Provide study card. Refer to KCMC	None

8.1 Participant groups 1 - 4

There are 4 participant groups.

Group 1: KCMC CASES
Group 2: CASES from Machame Hospital, Siha District Hospital and Huruma Hospital Rombo.
Group 3: CASES attending other clinics and hospitals in the Kilimanjaro Region
Group 4: CONTROLS, recruited at KCMC.

Case eligibility for groups 1, 2 and 3 are in 8.1
Control eligibility appears in 8.5.

8.2 Case Eligibility (for Groups 1, 2 and 3)

Eligible cases are:

- Aged 18 years or over at the time of visit
- Have confirmed or suspected oesophageal cancer (any histology)
- Patients presenting at ANY ward in the recruiting hospitals, with suspected or confirmed oesophageal cancer should be invited to participate in the study

Eligible patients who ***should*** be invited to participate include ALL patients:

- regardless of their stage at diagnosis/presentation to the clinic.
- regardless of race, country of origin, place of current or past residence, i.e. including those that live outside of the Kilimanjaro region.
- regardless of whether any curative treatment or palliative care will be offered to them.
- regardless of who is paying or of the ability to pay for medical care.
- regardless of whether they will ever return to the hospital for care or will follow referral advice.
- A patient who may have had preliminary surgery at another clinic, or had a confirmatory biopsy at another clinic and were referred for further treatment should be included.
- If a patient refuses at their initial visit, but changes their mind and would like to participate prior to treatment commencing at that hospital, they can be included.
- If a patient had a different cancer in the past, they should be included.

Note that although the scientific aims of the study concern ***the specific histology of squamous cell oesophageal carcinoma (ESCC)***, in this setting ALL oesophageal cancer patients should be included based on the clinical, imaging or endoscopic examinations prior to histology. For the patients who do not have a histological confirmation, approximately 85-90% of them will have ESCC. Including non-histologically confirmed cases in the analysis of risk factors will thus gain significant power even though a small number of such cases will in fact be incorrect inclusions (e.g. they are adenocarcinomas or non-malignant tumours). However the reduction in power through such small outcome misclassification will still result in a much larger gain in power than if we restrict the entire study to histologically-confirmed ESCC cases. For cases that undergo biopsy and their histology is non-malignant or non-ESCC, they will be excluded. All tumour-based work will necessarily have a biopsy and thus will only be conducted on cases with histologicaly-confirmed diagnoses.

8.3 Group 1: KCMC CASES

Cases at KCMC undergo the full study protocol, as both endoscopy and laboratory processing and storage facilities are available.

Summary:

The typical flow of a KCMC patient will be:

1. At the endoscopy unit, a patient with suspected esophageal cancer presents, as becomes evident during the endoscopy procedure.
2. The endoscopy team takes biopsy specimens (2 tubes, one tubes with formalin, one with RNAlater) per the protocols in Appendix A1 and A2.
3. The endoscopy team completes the ESCCAPE Presentation form (Appendix A4).
4. Upon completion of the endoscopy procedure, the endoscopy team informs the study interviewer (SI) that there is a new case.

Some KCMC patients with suspected oesophageal cancer will not undergo endoscopy (e.g. if they cannot afford it, or are too ill), but if, based on previous imaging or clinical symptoms alone, they are suspected to have oesophageal cancer (any histology), they should be invited to participate, in which instance the SI should be contacted and inclusion of the patient starts at step 6 below.

5. The SI collects the ENDOSCOPY form and the 2 biopsy tubes form the endoscopy unit.
6. The SI identifies where the patient is in the hospital and when they are in a comfortable situation, the SI should approach the patient, explain the study to them with the aid of the participant information sheet.
7. The SI opens the Mobenzi application "**CASES and CONTROLS: Enrol a new participant**", to obtain essential demographics and record whether the participant consents.
8. If the patient agrees to participate in <u>at least the questionnaire component</u> of the study, they are given the next available study ID. This will have the format 110XXX. There are 10 barcodes for each ID. The 10 barcodes ID labels (see picture below) on:

consent form,	presentation form;	urine container
study ID card	saliva spit	
2 biopsy tubes	2 blood tubes	1 spare

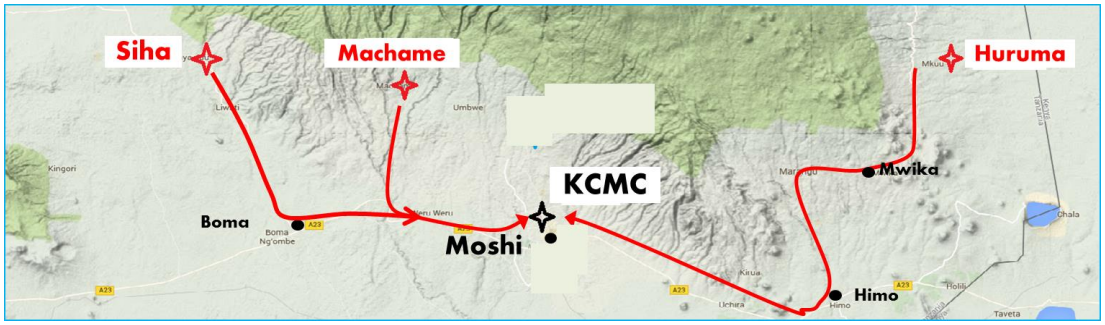
There is one spare label per ID. If a patient does not provide the blood, urine or does not have a tumor biopsy specimen, throw away their ID labels immediately (so as not to mistakenly use them on a different, incorrect, participant).

9. The m-application guides the SI through the remaining procedures consisting of:
    a. Full questionnaire on risk factors
    b. Taking a blood sample
    c. A 25ml urine sample
    d. Saliva spit
    e. Measure height and weight (appendix E1)
    f. Obtain a nail convexity score and dental fluorosis index.
10. At the end of the interview, the SI thanks the patient for their time, and gives them the study ID card in case they need to re-contact the SI.
11. The SI proceeds to complete entry of information in the endoscopy form.
12. The SI delivers the blood, urine, saliva and biopsy specimen to the pathology/KCRI laboratories for processing.

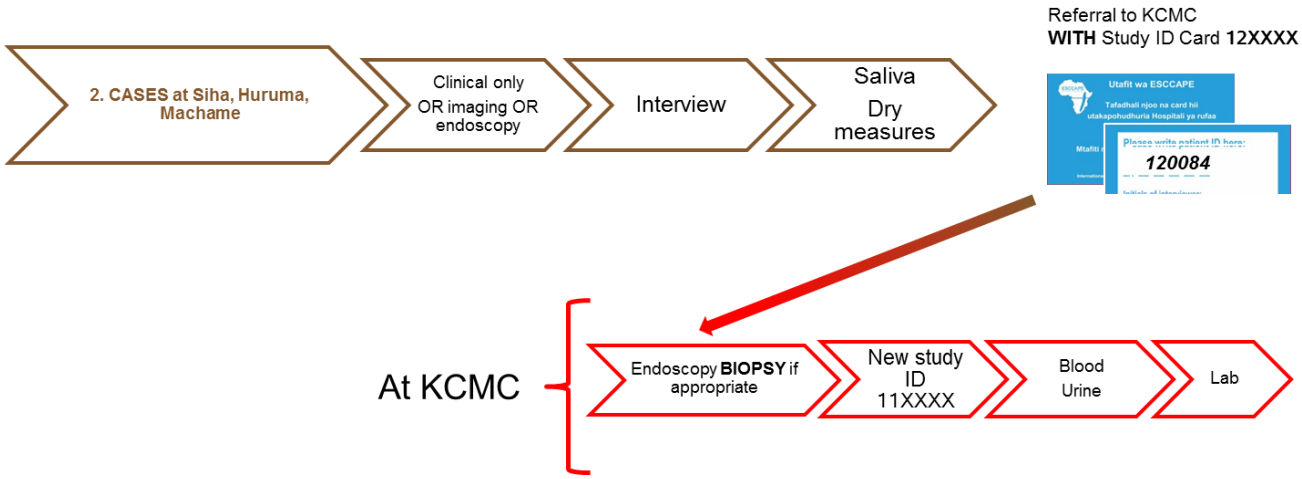
8.4 Group 2: CASES from Machame, Siha and Huruma Hospitals

These 3 hospitals have been identified as hospitals where a significant number of patients will be seen, who may or may not be able to reach KCMC. At the local hospital, the questionnaire is performed and saliva sample taken. The patient is given a study ID card and, if appropriate for their normal clinical care, they are referred to KCMC for endoscopy and to provide a blood sample and urine.

Locations of hospitals:



Summary:



Part A – When cases are at the district hospitals

The typical flow of ESCC patients recruited at Siha, Machame Hospital and Huruma Hospital will be:

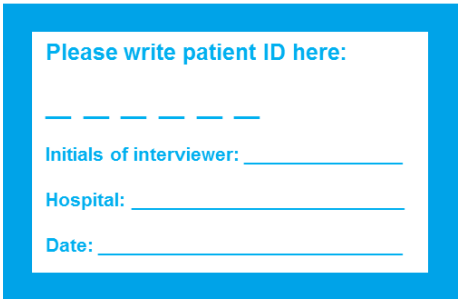
1. When a patient with suspected or confirmed oesophageal cancer presents, they will be eligible for the study. The basis of the suspected oesophageal carcinoma in these settings may be clinical-only (based on symptoms of dysphagia and weight loss), imaging (Barium swallow) or possibly endoscopy at Machame. So long as the patient has SUSPECTED or CONFIRMED oesophageal cancer, they are eligible.
2. The attending clinician completes the PRESENTATION form.

3. The attending clinician contacts the local study interviewer (SI) informing them of the presence of the eligible case and passes the PRESENTATION form to the SI.
4. The local SI ideally interviews the patient when they are ready at the clinic that day.
5. The local SI then approaches the patient, explains the study to them with the aid of the participant information sheet.
6. The SI opens the Mobenzi application "**CASES and CONTROLS: Enrol a new participant**", to obtain essential demographics and record whether the participant consents.
7. If the patient agrees to participate in <u>at least the questionnaire component</u> of the study, they are given the next available study ID, which has a format **12**0XXX. Place barcode ID labels on:
    a. Presentation form,
    b. Consent form,
    c. Study ID card,
    d. Saliva spit.

8. The m-application guides the SI through the remaining procedures consisting of:
    a. Full questionnaire on risk factors
    b. Saliva spit
    c. Measure height and weight
    d. Obtain a nail convexity score and dental fluorosis index.
    e. The SI selects **"NO"** when asked if a blood sample or urine sample are taken.

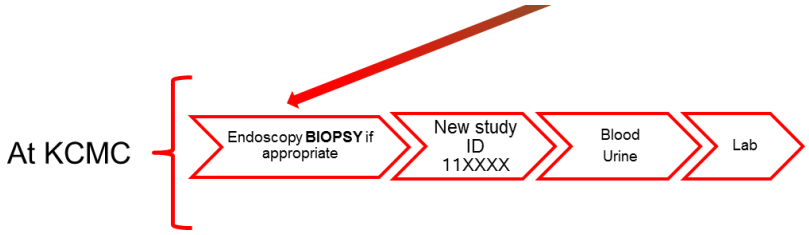
9. At the end of the interview, the SI thanks the patient for their time, and gives them the study ID card, making sure to write the patient's study number 12 XXXX on the back of it. If referral to KCMC is part of clinical care, the patient should be reminded to text the KCMC SI on the number printed on the card, before their visit to KCMC and to show their ESCCAPE study card when they get to KCMC and to arrange to meet with the KCMC SI when they are at KCMC.

Front of card **Back of card**



10. The local SI proceeds to complete entry of information in the presentation form.
11. The presentation form, consent form and saliva spit should be kept locally in a safe secure place. They will be collected every 2-3 months by the KCMC SI.

Part B – When cases who completed the questionnaire at the district hospital present at KCMC



1. If the KCMC study interviewer does not already know that the ESCCAPE patient is arriving, they should be contacted by any medical professional who realises that this is an oesophageal cancer patient who has already been enrolled in ESCCAPE.
2. The patient undergoes endoscopy if part of clinical care, with the endoscopy team completing the PRESENTATION form and taking biopsies.
3. When the KCMC SI speaks to the patient, they should ascertain clearly whether this is a NEW patient (in which instance they are part of Group 1), or whether they indeed have already completed the interview. If yes, the m-application form **<<<Patient is already an ESCCAPE case >>>** should be completed.
4. If the patient does not have their card, but the SI is sure that they have completed the questionnaire in Siha, Machame or Huruma, the patient can still be enrolled. The SI can first look up their previous ESCCAPE 12XXXX ID using the web-interface. Ideally the prior ID is known, but if it is not, the interview can continue and the SI should work with IARC and the local PI, or phone the local SIs to liaise and determine the patient's ID (12XXXX) from the district hospital
5. Now that because this patient has biospecimen at KCMC, they are now given a NEW KCMC ID (i.e. 11XXXX). **IT IS CRUCIAL THAT THE PERIPHERY ID IS LINKED TO THE KCMC ID FOR THESE PATIENTS, SO AS TO LINK THEIR QUESTIONNAIRE TO THEIR BIOLOGICAL SPECIMEN.** Take the next row of available KCMC barcodes (e.g. e.g. an ID 11XXXX). This time less of the labels will be used.

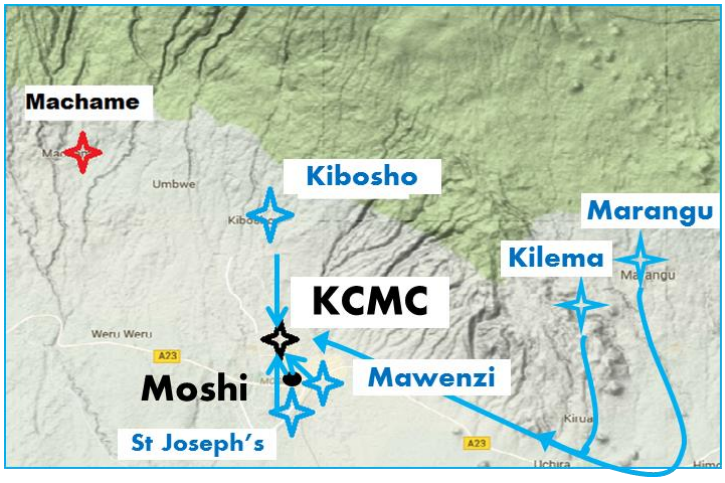
6. The patient is re-consented using the new KCMC ID, and is asked to provide two 5 ml blood samples and urine, placing the barcodes on each container.

Patient who was already interviewed and gave saliva at Machame/Huruma/ Siha are given a NEW KCMC. The crossed barcodes can be discarded.

7. The m-application works through the biospecimens collections. A saliva sample should have already been taken in the district hospitals. ONLY if the SI is convinced that it was not, for any reason, should they take it again.
8. The SI proceeds to complete entry of information in the endoscopy form.
9. The SI delivers the blood, urine, saliva and biopsy specimen to the pathology/KCRI laboratories for processing.

8.5 Group 3: CASES attending other clinics and hospitals in the Kilimanjaro Region

The following clinics and hospitals in near vicinity of KCMC will be centres where new suspected esophageal cancer patients will be provided study information only. These hospitals are: in Moshi town, Mawenzi Hospital and St. Joseph's hospital, Kibosho clinical, and to the east, Kilema and Marangu clinics.



1. If referral to KCMC for endoscopy would be part of the normal clinical care for a presenting patient with suspected oesophageal cancer, then log the patient name, gender and age. Give the patient the study information sheet and the ESCCAPE study card (no ID is to be written on it). The patient can contact the KCMC study

interviewer, whose mobile number is on the card, before they go to KCMC. If the patient

8.6 Group 4: CONTROLS, recruited at KCMC

8.6.1. Eligibility for Controls

Control participants are cancer-free persons, to which we will compare the cases. In ESCCAPE, controls will be recruited from across various outpatient hospital wards at KCMC (general surgery, orthopaedic surgery, outpatients and ophthalmology wards) or from visitors to the hospital. Patients who are eligible to be controls are those:

- Age 18 years or over at the time of being approached
- Do not have cancer now or in the past
- Are not being investigated for suspected cancer
- Are not attending the hospital with a digestive problem
- Have not already spent more than 3 nights in the hospital

Please note that controls should be recruited even if they don't come from the regular catchment area of the hospital, just as cases would be.

8.6.2 Age and sex distribution of controls

The age and sex distribution of the controls to be recruited is provided below. This distribution will ensure similarity of age and sex with the expected distribution in cases. Please note that if the study interviewers approach an eligible control and they are in an age-sex category in which a case has not yet been recruited, the eligible control should still be recruited (in the realistic expectation that a case will be recruited at some later date).

Age and sex distribution of every 100 controls to be recruited in ESCCAPE-Moshi

	MEN		**WOMEN**		
Age group (years)	**Target number**	**Count of actual numbers**	**Target number**	**Count of actual number**	total
20-29	2		2		
30-39	2		2		
40-49	7		5		
50-59	14		6		
60-64	12		5		
65-69	7		5		
70-74	10		4		
75+	11		5		
TOTAL	**68**		**32**		**100**

1.1. Consent for all participants.

The study nurse or study interviewer will be responsible for obtaining informed consent from every study participant. The following documents will be provided as part of the informed consent process:

- Study information sheet (in local language)
- Informed consent
- Copy of informed consent

The study nurse or interviewer will explain the purpose of the study and the participant's rights and roles in participation. The study information sheet will be presented before informed consent is obtained, and will be translated into the participant's local language (***Appendix A***). The informed consent will provide a brief overview of the study and will explain in detail what involvement in the study entails (***Appendix B***). Specifically, the informed consent will emphasize the person's rights as a study participant and the fact that the patient is allowed to withdraw from the study at any time.

One copy of the informed consent will be kept in the participant's study record. In addition, every participant will be given a copy of the informed consent for his/her personal records.

9. Data capture on tablet / phone

All data are capture on a tablet/phone using a tailor-made m-application for the ESCCAPE study. This application has been tested, but there will inevitably be additional changes need. Please email IARC with requests for changes. They can be made quickly, and all study interviewers can be requested to download the updated questionnaires, so that all interviewers are using the new same version in the field at all times.

There are three forms on the tablet:

- CASES and CONTROLS: Enroll new participant
- Cases: Pathology Results
- Patient is already an ESCCAPE case

CASES and CONTROLS: Enroll new participant

This is the main study questionnaire and it appears on the phone of every interviewer. It should be used for every participant who is approached to enter the study, i.e. for both cases and controls and for participants who wish to participate and those who do not.

The questionnaire is divided into sections:

a. Essentials – obtain the case/control status, age, gender, and which parts the participant agrees to do. If the participant **refuses**, this is recorded and the reasons for refusal are obtained and the questionnaire terminates.
b. Sociodemographic – The remaining sections are completed for both cases and controls.
c. Risk factors
d. Medical history – reported medical history. Duration of symptoms of the current dysphagia is only asked to cases.
e. Measurements – Oral health, height, weight, blood, urine, saliva, nail shape,

f. Basis of inclusion – enter findings from the Presentation form for cases, i.e. the findings from the Barium swallow or Endoscopy.
g. End

Cases: Pathology Results
This form mirrors that paper-based pathology form. The form is only visible to the KCMC interviewers, as only here will there be a pathology review.

Patient is already an ESCCAPE case
This is a short form that only appears on the tablet for the KCMC interviewer. This form is to be used when a patient has had their initial questionnaire and saliva sample at a district hospital, and then they come to KCMC for the endoscopy, urine and blood sample. At the start of this questionnaire, the interviewer is prompted to record the study ID (12XXXX) that is on the study ID card that the patient should have with them. Thereafter the patient is given a new study ID from KCMC (starting 11XXXX) and this new ID will be on all their biospecimen.

10.Questionnaires, Consent Forms and Participant Information Sheets

A blank copy is contained in the study manual.

APPENDICES Procedure and Biospecimen Protocols

A summary of protocols and specimen collected, and samples stored is provided below. Detailed appendices follow.

Collection	Subjects	Amount	Protocol – appendix index	Aliquots/ stored as	Final Storage	Comments for
A appendices: Specimen taken during ENDOSCOPY						
Tumour biopsy in formalin	Cases at KCMC	2-3 biopsies, oriented,	Appendix **A1 and A2**	Tumour slide and block	In study tumour cabinet drawer	
Tumour biopsy in RNA later	Cases at KCMC	2 biopsy specimen	Appendix **A3**	Single RNAlater tube as provided	-80°C freezer	
Report	Cases at KCMC, Siha, Machame, Huruma	-	Appendix **A4**	-	PRESENTATION reporting form	
B appendices: Specimen taken at PARTICIPANT INTERVIEW						
Urine	- Cases at KCMC - Controls at KCMC	25 ml minimum	Appendix **B1**	5 x 5 ml	-80°C	1 aliquot for PAH; 2 aliquots for trace elements; 1 storage; 1 other (e.g. cotinine or nitrates)
OragenDNA Saliva kit	Cases and Controls in all settings	2 ml saliva	Appendix **B2**	Single tube	Room temp.	Pt must not have consumed food, drank or smoked in the past 30 minutes
1 x 5 ml blood in EDTA tube	- Cases at KCMC - Controls at KCMC	1 x 5 ml	Appendix **B3**	Plasma: 4 or 5 x 0.5ml cryovials ~ 500 µl buffy coat in 1 cryotubes Red blood cells: 4 x 0.5ml cryovials	-80°C	
1 x 5ml blood in plain tube	- Cases at KCMC - Controls at KCMC	1 x 5 ml	Appendix **B4**	Serum: 4 x 0.5ml cryovial	-80°C	Keep serum. Discard RBC clot
C appendices: TUMOR TISSUE stored as FFPE blocks: Preparation and processing						
C1: Buffered formalin preparation, to be provided to the endoscopy team C2: Fixation C3: Tissue processing and preparation of FFPE blocks C4: Paraffin embedding C5: Sectioning of FFPE blocks C6: Staining of slides C7: Storage of FFPE blocks						
D appendix: PATHOLOGIST review						
Report	Cases	-	Appendix D1			
E appendices: SOPs for measurements taken by Study Interviewer during patient interview						
Height and weight measurements	Cases and controls	-	Appendix E1			
Fluorosis Index	Cases and controls		Appendix E2			
Nail curvature	Cases and controls		Appendix E3			

11. APPEDICES A-E

APPENDIX A1 – Endoscopy Procedure Protocol

*** Below is an adapted protocol from the Golestan GEMINI case-control study, amended to facilities and clinical protocols routinely followed at ESCCAPE local site.**

OBJECTIVES

1. Performing safe and informative diagnostic upper GI endoscopy on cases
2. Finding all visible abnormalities with acceptable accuracy
3. Finding pre-cancerous esophageal lesions for epidemiological, biological and therapeutic reasons
4. Providing high quality tissue biopsy of tumors and normal tissue for histopathological and molecular biological studies. A high quality specimen will enable all future molecular work.

PROCEDURE

1. The patient is brought into the endoscopy suite after the physician-assistant has filled out the study questionnaire and the patient has given an informed consent
2. In the endoscopy suite the nurse assistant re-explains what is going to be done in the endoscopy suite, calms him/her if necessary and directs him/her to lay on his/her left side on the endoscopy bed in a correct direction
3. The nurse assistant secures an open vein for the patient either with a butterfly or with an appropriate angiocath.
4. Oxygen with a nasal prong is administered continuously at a rate of 2-3 L/min, unless otherwise directed by the endoscopist.
5. The nurse assistant attaches the pulse oximetry detector to the patient and oxygen saturation and pulse rate are monitored continuously
6. A standard diagnostic video-endoscope is introduced through the mouth-piece located appropriately in the patient's mouth.
7. Start time is recorded
8. The scope is passed into the patient's esophagus and advanced toward his/her stomach and then the bulb and the second part of the duodenum under direct vision
9. Anatomical landmarks (upper esophageal sphincter (UES) and lower esophageal sphincter (LES)) and visible lesions are looked for and recorded with notion of their size and exact location. Length and shape of any columnar metaplasia in the lower esophagus is recorded. Erosive esophagitis is recorded according to the Los Angeles (LA) classification. This information is transmitted verbally to an assistant by the endoscopist during the procedure and the assistant records it on the Endoscopy Examination Form immediately. In addition, the endoscopist writes a separate formal report immediately after the procedure
10. Digital pictures are taken from any visible lesion during endoscopy. The pictures should document abnormal finding only.
11. If an area is not within the reach of the endoscope for any reason (e.g. tumoral obstruction, stenosis), please record this on the endoscopy form and the rest of the examination proximal to the obstruction performed
12. Take biopsies from the tumour or any suspicious lesion.
13. The procedure is then terminated, stop time recorded and the subject taken to the recovery room by the nurse assistant and cared for until he/she is completely conscious and alert. Then the subject and the next of kin are informed of the report of the endoscopy, a copy of the endoscopist's report is handed to them and directed to the follow-up desk where their next appointment is fixed. After all questions put

forward by the subject and the next of kin are answered appropriately, he/she may leave accompanied by the next of kin.

14. A well-equipped emergency trolley, containing all standard medication and equipment necessary for Advanced Cardiac Life Support (ACLS), should be at hand throughout the procedure and recovery
15. A cardiac defibrillator is available at bedside throughout the procedure and recovery.

Upper GI endoscopy carries a very small but definite risk to the patient. The endoscopy team should be prepared to prevent and if necessary handle these complications with adequate expertise. The following complications may be seen:

Aspiration (of oral contents or the sprayed Lugol's solution)
Hypoxemia
Respiratory failure
Cardiac arrhythmias
Esophageal perforation
Excessive bleeding from biopsy sites
Transmission of infection
Reactions to medications

The following are recommended to prevent these complications:

1. Maintain of a well-equipped emergency trolley with a functioning defibrillator in the procedure room all the time.
2. Question patients before the procedure concerning a history of bruising or bleeding or current use of aspirin or anticoagulant medication.
3. Question patients before the procedure concerning a history of reactions to iodine or lidocaine or contraindications to benzodiaipine use.
4. Position the patient properly so that the head and neck are about 20 degrees higher than the torso in the left lateral position, and maintain the patient in that position throughout the procedure.
5. Monitor all patients with a pulse oximeter during the procedure.
6. Provide nasal oxygen for all patients during the procedure.
7. Avoid sedation or decrease the dose of benzodiazepine used in elderly patients and those with underlying chronic cardiopulmonary disease.
8. If using Lugol's solution, limit its spraying to the minimal amount necessary, suction all of the solution from the stomach just after spraying and at the end of the procedure, and monitor patients' comfort during the procedure and apply titrating doses of sedation if necessary to avoid aspiration.
9. Avoid biopsying in patients with advanced portal hypertensive gastropathy or oesophageal or fundal varices.
10. Observe all patients for 30-60 minutes in the recovery room after termination of the procedure, until they are alert and ready to leave.
11. Wash the instruments appropriately (according to AGA guidelines), including disinfection with 2% Glutaraldehyde for 15-20 minutes between procedures to prevent transmission of infectious agents, especially viruses and mycobacteria.

12. The following are recommended to manage these adverse effects, if any of them happen:
13. At the first sign of serious cardiac or pulmonary complication, immediately halt the procedure and remove the endoscope.
14. Open and maintain the airway if respiratory depression occurs.
15. Use Basic Life Support, or if necessary, Advanced Cardiac Life Support procedures to convert serious cardiac or pulmonary complications.

16. Promptly reverse the action of Benzodiazepine with IV Flumazenil (an initial bolus of 0.2mg over 30 seconds followed by 0.5mg boluses over 30 seconds at 2 minute intervals, if necessary, not to exceed a total dose of 3-5mg).
17. Observe the patient for at least 2 hours in the recovery room after reversal of an adverse event, if discharging the patient.
18. In the case of a perforation, attempt to insert a covered esophageal stent, if clinically indicated. Support the patient with IV fluids, antibiotics, and appropriate X-rays in the ICU, in close collaboration with an expert surgical team.
19. In the case of excessive bleeding, achieve hemostasis with Argon Plasma Coagulation.

Reference: Gastroesophageal Malignancies In North of Iran (GEMINI) Protocol, Digestive Disease Research Center (DDRC), Tehran University of Medical sciences (TUMS), Tehran, Iran, 2003

APPENDIX A2 - Esophageal Biopsy Tissue Collection and Processing

Taking representative parts of tissue for routine diagnosis must have the priority to getting the material for the tissue bank. Research aims should not compromise routine diagnosis for the patients.

All procedures should be carried out in accordance with the local codes of practice.

All tissues must be handled as if potentially infectious.

4 or 5 biopsies of the tumour should be taken using the appropriately-sized forceps.

Two to three biopsies are taken from the tumour. **Each biopsy sample is delivered to the assistant in endoscopy room who should orient and spread the specimen over a filter paper, mucosal side up,** and then place it, with the paper, in two bottles of 10% buffered formalin. No more than three biopsies should be placed in one tube.

For placement in RNA later tube: Two biopsies are taken from the tumor and are placed in a single RNAlater® tube.

APPENDIX A3 - Protocol for Biopsy Preservation in RNAlater®

RNA*later*® is a commercial aqueous, non-toxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA. RNA*later*® eliminates the need to immediately process tissue samples or to freeze samples in liquid nitrogen for later processing.

In ESCCAPE, a 1.5 ml RNAlater tube is being used (as shown).

1. Place 2 tumour biopsies directly into the RNAlater tube at the time of endoscopy.
2. Label RNA*later*® tubes with a QR barcode for temporary identification.
3. Log the tube at the laboratory and label with a cryolabel suitable for -80 ˚C.
4. Samples should be left overnight in a fridge (2˚C to 8˚C). Record the time when collected from the participant and when placed in the fridge in the Biospecimen log in appendix B5.

The following morning, the reagent **should first be drained away and the tube immediately stored in a -80 freezer.** Enter storage location in the computerized database system and enter the time of freezing in Biospecimen log in appendix B5.

APPENDIX A4 - Presentation form (CASES)

The physician treating the patient should complete the Presentation form for every suspected oesophageal carcinoma and alert the study interviewer of a new patient's presentation. This form should be handed to the research assistants, alongside the tumor biopsies.

Place ESCCAPE ID barcode here

ESCCAPE case inclusion: PRESENTATION Form

Please complete for patients aged 18 years and over with confirmed or suspected esophageal cancer.

Date patient presents: __ __ / __ __ / __ __ __ __ day/month/year

SURNAME:

First name (s):

Date of birth: __ __ / __ __ / __ __ __ __ day/month/year

Hospital number:

ALL PATIENTS

1. REFERRAL ROUTE

- Direct presentation to hospital ☐
- Referral by primary clinic ☐
- Referral from district hospital ☐
- Other ☐

2. SYMPTOMS on presentation (multiple)

- Solid dysphagia ☐
- Liquid dysphagia ☐
- Dyspepsia ☐
- Hematemesis ☐
- Weight loss ☐
- Axillary nodes ☐
- Metastases ☐
- Palpable spleen ☐

Metastatic site: _____

Was endoscopy performed?

- Yes ☐
- No ☐ → Turn over

3. BASIS OF DIAGNOSIS (multiple)

- Clinical ☐
- Imaging/Barium swallow ☐
- Surgery ☐
- Cytology ☐
- Histology of primary ☐
- Histology of metastases ☐
- Endoscopy finding ☐

ENDOSCOPY Results

Is esopahgeal carcinoma suspected? Yes ☐ No ☐

Is a histopathology report requested? Yes ☐ No ☐

Length of esophagus (canines to LES, if not blocked): _____ cm

Location of suspect esophageal tumour (canines to tumor): _____ cm

TUMOR LOCATION:

- Cervical (proximal) esophagus (15-19 cm) ☐
- Upper thoracic (20-24 cm) ☐
- Middle thoracic (25-29 cm) ☐
- Lower thoracic (30-40cm) ☐
- Esophageal-gastrojunction ☐
- Other (please specify) ☐ _____

Tumor maximal diameter (approx. visual assessment): _____ cm

Tumor biopsies taken: Number in formalin (minimum 3-4): ☐ ***Please orientate biopsies.***

Number in RNAlater (minimum 1-2): ☐

Other esophageal pathologies present (if any)

None	☐	Esophageal varices	☐
Esophagitis	☐	Stricture	☐
Candidiasis	☐	LES	☐
Erosion	☐	Stricture	☐
Erythema	☐	Other, please specify	☐

Problems during endoscopy

None	☐
Tumor blocks lumen	☐
Hypoxia	☐
Cardiac arrhythmia	☐
Esophageal performation	☐
Excessive bleeding	☐
Other, please specify	☐ _____

BARIUM SWALLOW

Was barium swallow performed? Yes ☐

No ☐

Indication for barium swallow

Dysphagia	☐
GERD	☐
Possible hiatus hernia	☐
Gastric pain	☐
Vomiting	☐
Possible fistula	☐
Cannot endoscope	☐
Other, please specify	☐ _____

Is esopahgeal carcinoma suspected? Yes ☐

No ☐

TUMOR LOCATION:

Cervical (proximal) esophagus (15-19 cm)	☐
Upper thoracic (20-24 cm)	☐
Middle thoracic (25-29 cm)	☐
Lower thoracic (30-40cm)	☐
Esophageal-gastrojunction	☐
Other (please specify)	☐ _____

Tumor maximal diameter (approx. visual assessment): _____ cm

COMMENTS **Physician's name:** _____

APPENDIX B1 - Urine

A minimum **25 ml urine sample** is collected for each of the cases, controls and accompanying neighbours. It will be aliquotted into 5 x 5 ml cryotubes. This amount will be needed for trace elements (2 tubes), PAH (1 tube), archive (1 tube) and other (e.g. nitrates, cotinine).

1. Materials needed:

Urine collection cup with wide-mouth and leak-proof screw cap (50 ml or 100 ml plastic cup, sterile), known to be trace element free
Pre-printed freezer-appropriate study labels
5 cryovials (5 ml), known to be trace element free
Transfer pipette
Pipette tips
Powder-free lab gloves
Boxes with grids to hold 5 ml plastic tubes in storage freezer

2. General instructions

1. There is no special instruction for fasting or for special diets for the participant, however it is recommended to collect the urine after a period when the participant has not drank a lot, as the urine would be diluted. If possible, collect urine in the morning.
2. The minimum required volume of urine is 25 ml. Specimens will be deemed unacceptable if it is suspected that they have been contaminated by dust, dirt, or other contaminants resulting from improper handling. Dust and other contaminants can greatly affect the quality of urine specimens, and research assistants should therefore be highly aware of possible specimen contamination.
4. The study labels for the urine collection cup should be affixed to the container before the specimen collection has begun.

C. Patient urine collection

1. The pre-labelled urine collection cup should be handed to the participant with the cap secured.
2. Each participant should be instructed to do the following before urine collection:
3. Wash hands with soap and water when possible.
4. Do not open the collection cup until just before urinating.
5. Collect at least 25 ml of urine in the cup (indicate this volume on the outside of the container to the participant). After urinating in the cup, immediately recap the filled container tightly. Do not touch the inside of the cup or cap at any time.
6. The researcher should ensure that the pre-labelled urine collection cup lid is secure.
7. Record the collection time in the Biospecimen log (see appendix B5/B6).

It is most important that the inside of the container and the cap not be touched or come into contact with any parts of the body or clothing or external surfaces. Exposure to air should be minimized.

D. Storage

1. Aliquot the urine into 5 x 5 ml cryotubes and label appropriately. Store at -80°C.
2. Record the freezer storage time in the Biospecimen log (see appendix B5/B6).

APPENDIX B2 - Saliva spit (All cases and controls)

Esophageal cancer patients are asked to provide a saliva spit. One OrageneDiscover kit is used per patient.

Check that the kit date is still valid.

Verify that the patient has not eaten, drank or smoked with in the past 30 minutes.

Take the saliva sample using the manufacturer's instructions below.

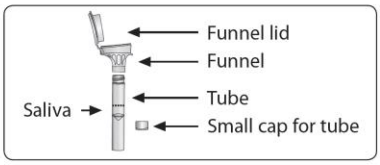
Once the sample is taken, label it with the participant's study ID, date and store in a **secure locked cabinet, out of direct sunlight, at room temperature.**

Record the collection time in the Biospecimen log (see appendix B5).

ORAgene®•DISCOVER

REF OGR-500

For Research Use Only
Not for use in diagnostic procedures



Collection precautions:

Do NOT eat, drink, smoke or chew gum for 30 minutes before giving your saliva sample.

Do NOT remove the plastic film from the funnel lid.

This product is designed for the collection of human DNA from saliva samples.

Contents: Kit contains stabilizing liquid.

Warnings and precautions: Wash with water if stabilizing liquid comes in contact with eyes or skin. Do not ingest. See MSDS at www.dnagenotek.com.

Small cap, choking hazard.

Storage: 15°C – 30°C

Summary and explanation of the kit:

Oragene•DISCOVER is a self-collection kit that provides the materials and instructions for collecting and stabilizing saliva specimens.

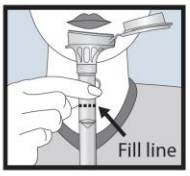
PD-PR-00279 Issue 1/2012-10

USER INSTRUCTIONS

Read all instructions prior to collection

Procedure:

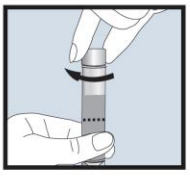
Most people take between 2 and 5 minutes to deliver a saliva sample following steps 1 to 5.



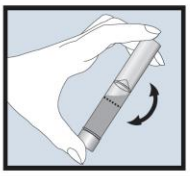
1 Spit into funnel until the amount of liquid saliva (not bubbles) reaches the fill line shown in picture #1.

2 Hold the tube upright with one hand. Close the funnel lid with the other hand (as shown) by firmly pushing the lid until you hear a loud click. The liquid in the lid will be released into the tube to mix with the saliva. Make sure that the lid is closed tightly.

3 Hold the tube upright. Unscrew the funnel from the tube.



4 Use the small cap to close the tube tightly.



5 Shake the capped tube for 5 seconds. Discard or recycle the funnel.

Label legend:

	Consult package insert
	Collect saliva by (Use by)
REF	Catalog number
D	Industrial Design Patent
	Caution, consult instructions for use
15°C – 30°C	Storage instructions
	Manufacturer

For Research Use Only
Not for use in diagnostic procedures

Superior samples • Proven performance

Made in Canada by DNA Genotek Inc.
2 Beaverbrook Road, Ottawa, ON, Canada K2K 1L1
Toll-free (North America): 1.866.813.6354
Tel.: 613.723.5757 • Fax: 613.723.5057
www.dnagenotek.com • info@dnagenotek.com

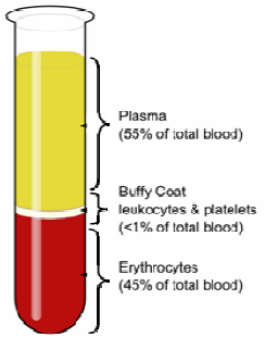
DNA genotek
www.dnagenotek.com

APPENDIX B3 – Blood collection in EDTA tubes (KCMC cases and controls)

Blood specimens need to be processed as soon as possible, ideally within 2 hours, but in extreme conditions up to a maximum of 12 hours. This is critical for time-sensitive samples for protein studies, for example.

Materials

- 1 x 5 ml vacutainer EDTA tube with screw tops, for the collection of plasma and buffy coat and red blood cells
- 11 x 0.5 ml cryovials per participant
- Pipettes
- Centrifuge
- cryoboxes



Procedure

1 One 5 ml EDTA vacutainer tube of blood is obtained by venepuncture from the consented participant. After blood withdrawal, vacutainers will be immediately protected from direct light and kept at 5-10 °C. Record blood collection time on the Biospecimen log (appendix B5).

2 At the processing lab, record the start of processing time on the Biospecimen log (appendix B5).

3 Spin the EDTA vacutainer at 800xg for 10 minutes, if possible spin at 4°C. This will separate out the three layers as in the figure: plasma at the top, a thin greyish layer representing the buffy coat fraction in the middle and the red blood cells which is the dark red layer at the bottom.

4 **Plasma:** Using a scientific pipette, aspirate off the plasma layer (about 50-60% of the sample) into 0.5ml cryovials. Continue until all the plasma has been removed, but without disturbing the buffy coat layer. A total of 4 or 5 cyrovials (0.5ml) should be obtained.

Store in a -80°C freezer.

Record freezer storage time of the plasma on the Biospecimen log (appendix B5).

1. **Buffy coat cells:** The buffy coat is a thin, greyish-white layer of white blood cells (leukocytes and lymphocytes) and platelets covering the top of the packed red blood cells after 800xg centrifugation (from EDTA/ACD containing blood tubes).

After having spun the blood and removed the plasma, use a pipette with an adjustable volume to take off the entire buffy coat layer, including about 100µl of plasma. Be careful not to lift red cells.

Aliquot the buffy coat from the pipette into 2 labelled cryovials of 0.5ml.

Place in –80°C freezers.

Record freezer storage time of the buffy coat on the Biospecimen log (appendix B5).

Red blood cells (RBCs): Aliquot the remaining RBC layer into 4 labelled 0.5ml cryovials and store them at –80°C. Record freezer storage time of the RBC on the Biospecimen log (appendix B5).

APPENDIX B4 – Blood collection in plain tubes (KCMC cases and controls)

Materials

- 1 x 5 ml plain vacutainer (no anticoagulant) tube with screw tops, for the collection of serum
- 4 x 0.5 ml cryovials per participant
- Pipettes
- Centrifuge
- Cryoboxes

Procedure

1 One 5 ml plain vacutainer tube of blood is obtained by venepuncture from the consented participant. After blood withdrawal, vacutainers will be immediately protected from direct light. Record blood collection time on the Biospecimen log (appendix B5).

Clotting: The blood is collected into tubes without addition of anticoagulants. Then two phases become distinguishable within the tube: a solid phase containing fibrin and cells at the bottom, and a fluid phase containing the serum. This clotting process should be completed after 30 minutes at room temperature, the time necessary for blood clot formation, after which the process described below starts.

2 Spin blood at 1500xg for 10 minutes at room temperature.

3 Aliquot portions of supernatant (serum) into 4 labelled 0.5ml cryovials.

4 Transfer to -80°C freezer.

5 Record freezer storage time of the RBC on the Biospecimen log (appendix B5).

6 The remaining clot can be discarded.

APPENDIX C Tumour tissue preparation and processing

Appendix C includes protocols for:

- **C1: Buffered formalin preparation, to be provided to the endoscopy team**
- **C2: Fixation**
- **C3: Tissue processing**
- **C4: Paraffin embedding**
- **C5: Sectioning**
- **C6: Staining**
- **C7: Storage**
-

C1: Buffered formalin preparation

10% neutral buffered formalin:

To make 1 L of working solution of 10% neutral buffered formalin from stock solution of formaldehyde 40%, follow the instruction below:

Formaldehyde (37-40%) ---------- 100 ml
Distilled water ---------- 900 ml
Sodium dihydrogen phosphate, monohydrate (NaH_2PO_4.H2O) ---------- 4.0 g
Disodium hydrogen phosphate, anhydrous (Na_2HPO_4) ---------- 6.5 g
Mix to dissolve.

C2: Formalin fixation

Formalin fixation is standard practice in most routine histopathology laboratories. The recommended concentration is 10% neutral buffered formalin. It is important that the fixative is buffered to avoid the formation of formaldehyde pigment on blood rich tissues and also improve the yield of nucleic acids.

Tissue specimens should not be bigger than 1.5 x 1 x 0.5 cm.

Specimens will be fixed in fresh 10% neutral buffered formalin (see the preparation protocol above) in 10 times volume of the tissue, for a minimum of 4 hours and a maximum of 24 hours, after which time they will be embedded in paraffin following conventional techniques.

All reagents should be DNAse, RNAse free (e.g. prepared using DEPC, distilled or millipore water).

Fixation media containing Picric acid (e.g. Bouin's) should be avoided as this compound interferes with subsequent PCR analysis of extracted nucleic acids.

The biopsy samples fixed in formalin are processed according to the procedure described by Prophet (see the reference list).

The processed samples are blocked in paraffin considering the anatomic orientation of the tissue.

C3: Tissue processing and preparation of FFPE blocks

Once fixation is terminated, place the biopsies in embedding cassettes, properly labelled. Process by an automation or manual method, with the following order;

1- Ethyl alcohol 90 1 hour (gradual dehydration)
2- Ethyl alcohol 96, two changes, 1 hour each
3- Ethyl alcohol 100, three changes, 2 hours each
4- Xylene (or xylene substitue), 3 changes, 1 hour each
5- Paraffin (55-60 °C), two changes, 1.5 hours each

Total time=15 Hours

Program the automation device in a manner to avoid leaving the tissues in hot paraffin for long time before embedding in paraffin.

C4: Paraffin embedding

1. Embed tissue into paraffin blocks. Clean up the working place to avoid embedding unwanted material except the tissue.
2 Do not embed more than three biopsies in one block.
3 Be careful to embed all biopsies at the same level by gently forcing them to attach the bottom of the block. While paraffin is still liquid, there is a risk of displacement of tissues. Avoid this by observing the process till solidification of paraffin.
4 Label the paraffin blocks properly.

C5: Sectioning

Cut at 3-5µm, using a rotary microtome.
Place obtained paraffin ribbons in water bath at about 40-45ºC
***keep the water bath clean and free from remnants of the previous sectioning.*
Mount sections onto slides.
Allow sections to air dry for 30 minutes and then bake in 37-45ºC oven overnight. They may be dried at 60°C for 15 minutes if they are required the same day.

C6: Hematoxylin & Eosin (H&E) staining

Deparaffinise sections in 2-3 changes of xylene, 10 minutes each

Hydrate in 2 changes of 100% ethanol for 3 minutes each, 95% and 80% ethanol for 1 minute each
Rinse in water
Immerse the slides in Hematoxylin for 5 min (subjective adaptation is possible)
Rinse in water.
Immerse in acid-alcohol solution (few dips for removal of excess dye).
Rinse in water.
Immerse the slides in lithium carbonate (few dips for buffering the acidity and fixation of hematoxylin).
Rinse in water.
Immerse the slides in Eosin for 3-5 minutes (subjective adaptation is possible).
Dehydrate through ethanol 96, two changes, 5 min each.
Xylene, at least 3 changes, 3-5 min each.
Mount the slides with 24x50 mm coverslips.

C7: Storage of FFPE blocks

FFPE blocks and sections mounted on slides can be stored at room temperature
Prevent exposure of blocks to sun or extreme temperature variance
Store blocks in moisture resistant cardboard boxes or plastic storage boxes.

All the slides are examined microscopically for preparation of the official pathology reports and recording of the details of pathologic findings using the formats and coding system developed for this study by an authorized pathologist.

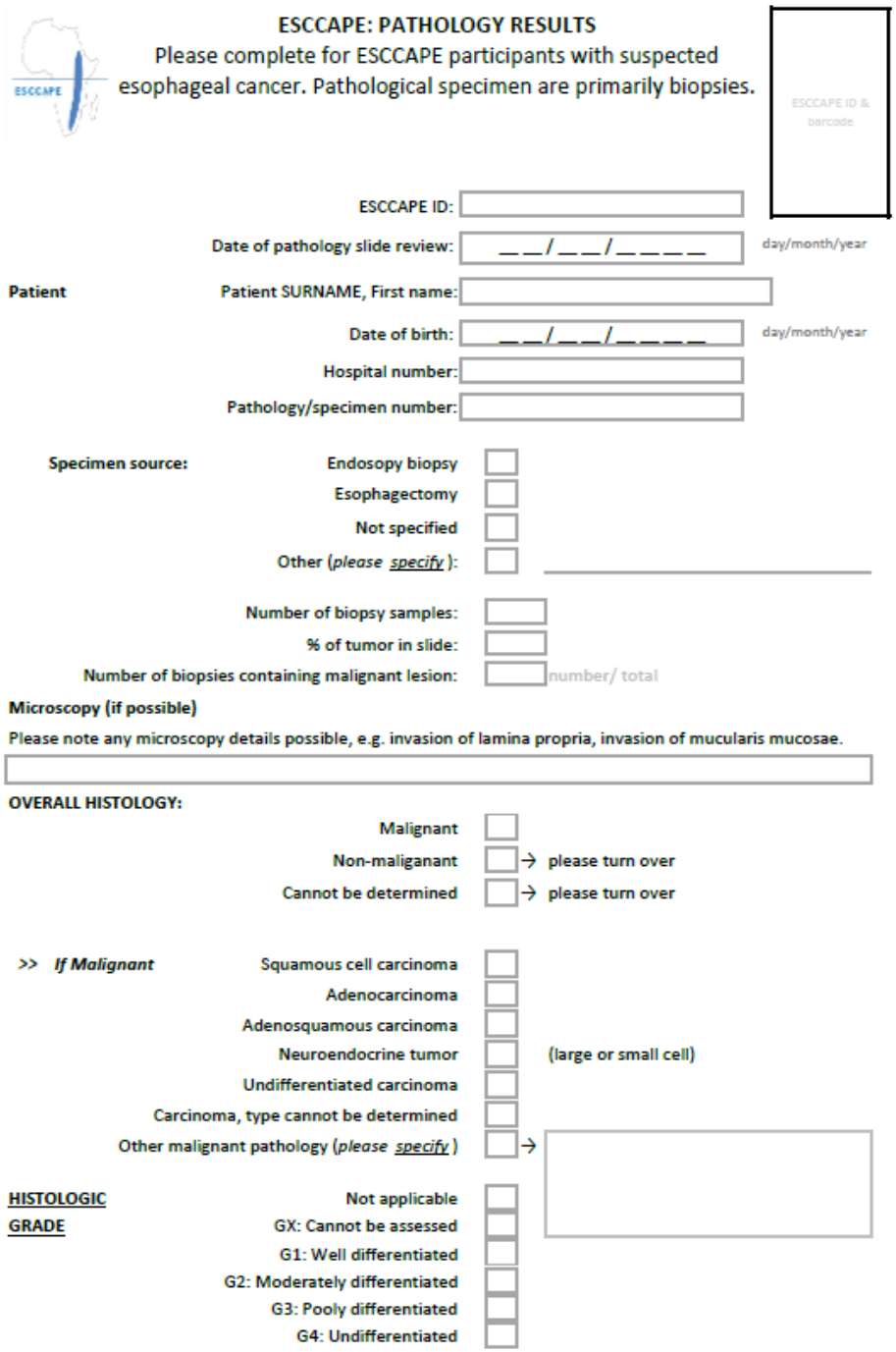
Re-examination of all the slides containing tumor tissue and one out of ten non-tumor cases chosen randomly is performed as a second look by another authorized pathologist as a measure of diagnostic quality control. Discrepant diagnoses are resolved by joint review

References:

TUBAFROST instructions ((http://www.tubafrost.org)
Gastroesophageal Malignancies In North of Iran (GEMINI) Protocol, Digestive Disease Research Center (DDRC), Tehran University of Medical sciences (TUMS), Tehran, Iran, 2003
International liver Cancer Study (ILCS) Protocol, International Agency for Research on Cancer (IARC), Lyon, France, 2007
Common Minimum Technical Standards and Protocols for Biological Resource Centers Dedicated to Cancer Research, IARC working group, Lyon, France, 2007
Prophet E.B., Mills B., Arrington J.B., et al. Laboratory Methods in Histotechnology. Armed Forces Institute of Pathology, 1994

Appendix D: PATHOLOGY FORM

Stained slides are prepared for the pathologist's review, and the completed endoscopist's form should be provided to the pathologist during their evaluation. The pathologist is asked to complete the following form and return it to the study research assistants, who will enter it into the tablet.



ESCCAPE: PATHOLOGY RESULTS

Please complete for ESCCAPE participants with suspected esophageal cancer. Pathological specimen are primarily biopsies.

ESCCAPE ID & barcode

ESCCAPE ID:

Date of pathology slide review: __ __/__ __/__ __ __ __ day/month/year

Patient

Patient SURNAME, First name:

Date of birth: __ __/__ __/__ __ __ __ day/month/year

Hospital number:

Pathology/specimen number:

Specimen source:

- Endosopy biopsy ☐
- Esophagectomy ☐
- Not specified ☐
- Other (*please specify*): ☐ _____

Number of biopsy samples: ☐

% of tumor in slide: ☐

Number of biopsies containing malignant lesion: ☐ number/ total

Microscopy (if possible)

Please note any microscopy details possible, e.g. invasion of lamina propria, invasion of mucularis mucosae.

OVERALL HISTOLOGY:

- Malignant ☐
- Non-maliganant ☐ → please turn over
- Cannot be determined ☐ → please turn over

>> *If Malignant*

- Squamous cell carcinoma ☐
- Adenocarcinoma ☐
- Adenosquamous carcinoma ☐
- Neuroendocrine tumor ☐ (large or small cell)
- Undifferentiated carcinoma ☐
- Carcinoma, type cannot be determined ☐
- Other malignant pathology (*please specify*) ☐ →

HISTOLOGIC GRADE

- Not applicable ☐
- GX: Cannot be assessed ☐
- G1: Well differentiated ☐
- G2: Moderately differentiated ☐
- G3: Pooly differentiated ☐
- G4: Undifferentiated ☐

>> If non-malignant

Benign tumor (e..g. leiomyoma, *specify*) ☐ →

No evidence of malignancy ☐

Esophagitis ☐ →

Other non-malignant pathology (*please specify*) ☐ →

Specimen inadequate for evaluation (*specify reason* ☐ →

you like the slide to be reviewed by another pathologist? ☐ Yes ☐ No

Pathologist: Name: _____

Signature: _____

Please write any other comments or queries.

Any comments to feedback to the study would be very useful (e.g. to the ESCCAPE team, to endoscopy, on pathology, classification, or on the format of this form). Thank you.

Appendix E1: HEIGHT AND WEIGHT MEASUREMENTS

Height and weight measurements are made at the end of the questionnaire, for cases and controls. The participant should be asked to remove as much outerwear as possible. Regardless of the clothing worn, the participant should be asked to remove her shoes and will be measured barefoot (or in tights/stockings). Additionally, the participant should be asked to empty their pockets and remove heavy or bulky jewellery, or other objects. The participant should be asked to remove eyeglasses, hair barrettes, and if possible anything that may impair accurate height and weight measurements from being obtained:

Height Measurement
For the measurement of standing height, the back, scapulae and buttocks should be in contact with the vertical board if possible, or whichever part of the body touches the board first. Weight should be evenly distributed on both feet, and legs together (ankles or knees together, whichever comes together first, often they will come together simultaneously).

The participant should stand erect (stand up straight and look straight ahead). Position the head in the Frankfort Horizontal Plane. In this position, an imaginary line can be drawn from the bottom of the eye socket (orbital margin) to the external opening of the ear (external auditory canal).

Ask the participant to inhale deeply and hold her breath WHILE MAINTAINING the head and body in the same position. Sometimes the head will lift (or body rise onto the toes) when taking a deep breath, if this happens, re-position the body and head before taking the measurement.
The moveable headpiece is brought onto the upper most point on the head with sufficient pressure to compress the hair. The measurement is recorded to the nearest 0.1cm, or nearest mm, depending on the stadiometer used.

Weight Measurement
For the measurement of weight, the participant should stand still over the centre of the scale with body weight evenly distributed between both feet. Arms should be hanging freely by the sides of the body, with palms facing the thighs. The participant should hold her head up, and face forward. Weight is recorded to the nearest 0.1 kilograms.

Appendix E2: Fluorosis Index Score

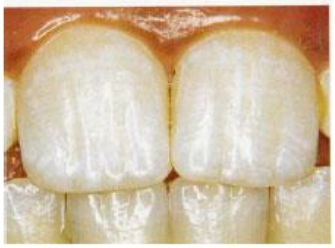
Fluoride lesions often affect the tips of cusps or incisal edges; the lesions often appear as lines which follow the incremental lines in enamel and shade imperceptibly into the surrounding normal enamel. Bilateral pairs of teeth are affected (e.g., 11 and 21). Cuspids, bicuspids second and third molars are most commonly affected. It is rarely observed on lower incisors and almost never in the primary dentition. The mild lesions are commonly described as having papery white appearance. The enamel surface is smooth to an explorer. Marks are often invisible under strong light.

Because differential diagnosis of fluorosis is difficult in the mildest cases, **if in doubt, score 0.** If you are convinced that the lesions you are viewing are truly due to fluoride, score according to the worst **bilateral pair of homologous teeth that you can see**. If only one tooth (not a pair of teeth) is involved, do not score as a fluorotic lesion.

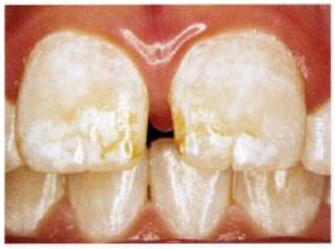
- ☐ 0 NORMAL - no fluorosis [0]
- ☐ 1 VERY MILD - narrow white lines, no pits [1]
- ☐ 2 VERY MILD - pronounced lines at ridge, discoloration area < 2 mm [2]
- ☐ 3 VERY MILD/MILD -merging cloudy areas [3]
- ☐ 4 MODERATE - cloudy or chalky area on whole tooth [4]
- ☐ 5 SEVERE – small pits - whole tooth discolored, PITS < 2mm"; [5]
- ☐ 6 SEVERE – small pits - whole tooth discolored, PITS regularly arranged"; [6]
- ☐ 7 SEVERE – large pits - loss of enamel in less than half of tooth [7]
- ☐ 8 SEVERE – large pits - loss of enamel in more than half of tooth [8]
- ☐ 9 (loss of most of enamel [9]
- ☐ 88 - not possible to evaluate [88]
- ☐ 99 - no teeth [99]

TF-indeksen

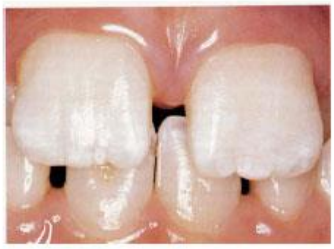
TF 1



TF 3

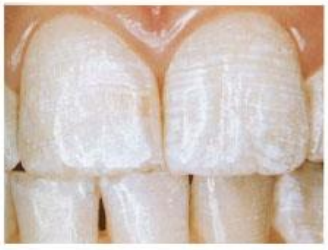


TF 1

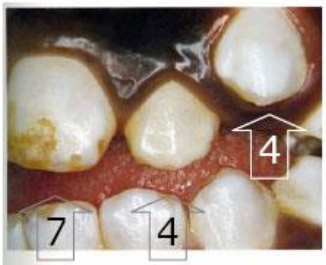


TF 4/5

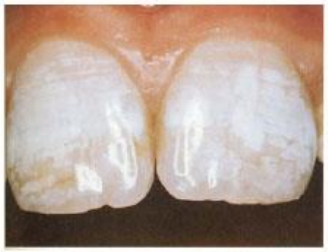
TF 2



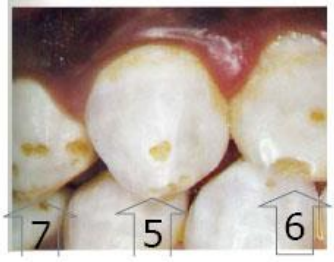
TF 4/7



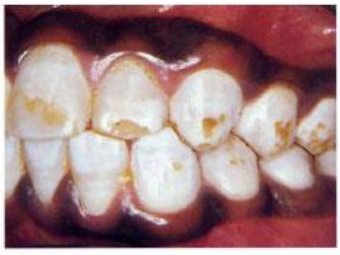
TF 3



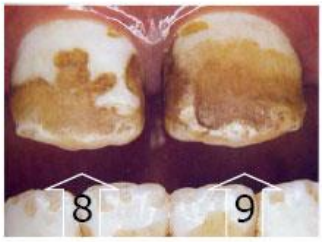
TF 5-7

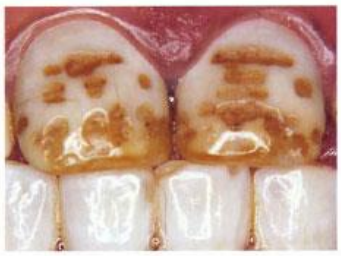


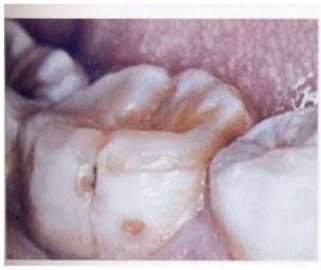
TF 7



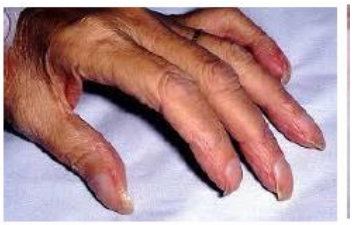
TF 8/9







Appendix E3: Nail Curvature Score

1=normal nail	 Normal nail
2=convex nail	 Convex nail
3=concave nail	 Concave nail
4=not possible to evaluate	