Central Review of Pathology Specimens

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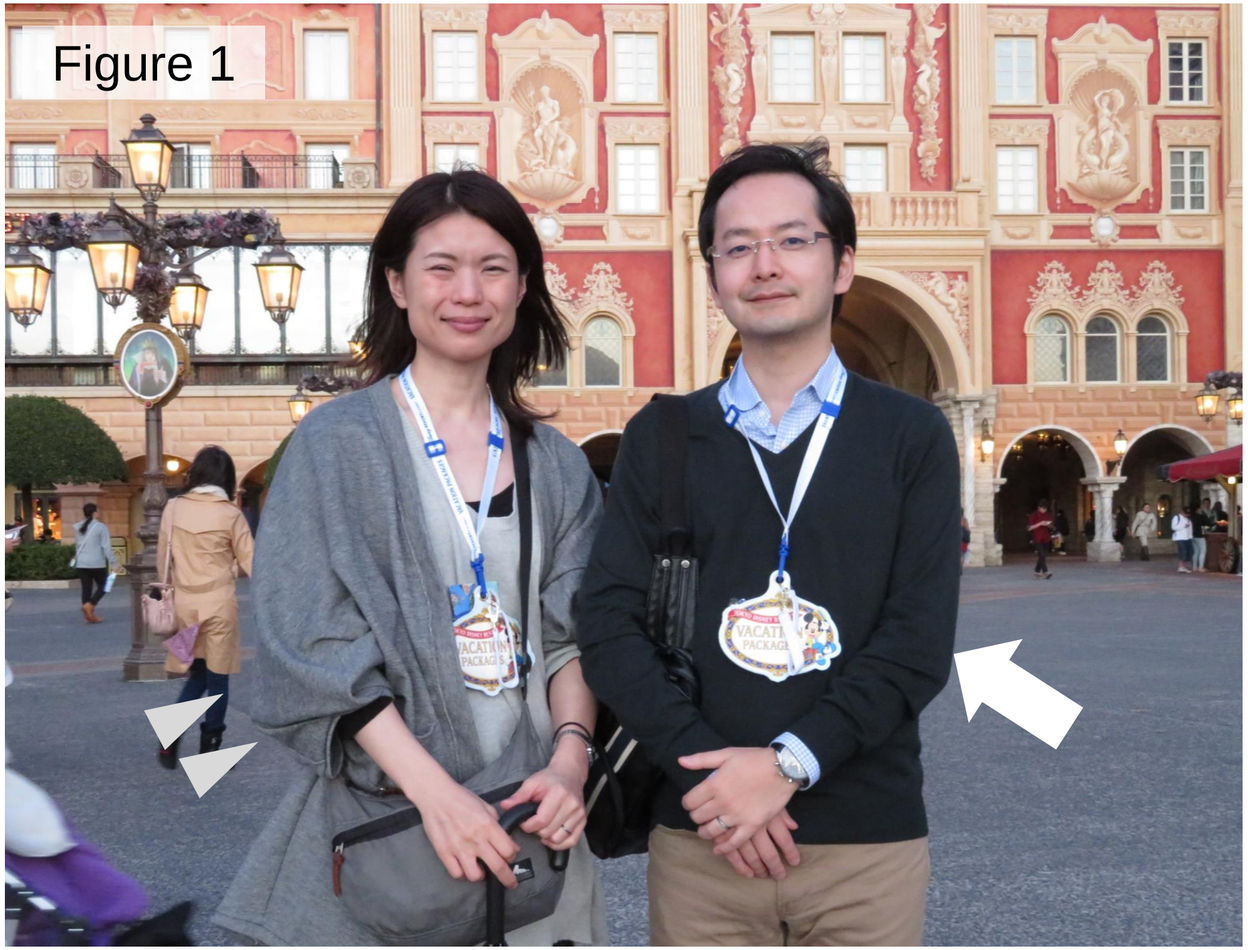
ASIAN CLINICAL TRIALS NETWORK
FOR CANCERS PROJECT

Figure 1



Hiroshi Yoshida, M.D., Ph.D. (white arrow)

Staff Pathologist

Department of Diagnostic Pathology

with his wife (arrow heads) in Tokyo Disney Sea

Position/Title	Name and Location of Institution	Duration
Current Staff Pathologist / Dept. of Diagnostic Pathology	National Cancer Center Hospital (Chuo-ku, Tokyo, Japan)	Nov/2011- Present
Previous Attending Physician / Dept. of Pathology	Asahi General Hospital (Asahi, Chiba, Japan)	Apr/2010- Oct/2013
Previous Resident / Dept. of Medical Oncology	National Cancer Center Hospital (Chuo-ku, Tokyo, Japan)	Apr/2008- Mar/2010
Previous Resident / Dept. of General Internal Medicine	Kure Medical Center (Kure, Hiroshima, Japan)	Apr/2005- Mar/2008

Contents

- Outline of MASTERKEY-Asia
- Efforts of the Department of Diagnostic Pathology
- Preparation of pathology specimens and quality check of submitted samples
- Pathology review

ATLAS

MASTER KEY ASIA

MASTER KEY Asia project

Marker Assisted Selective ThErapy in Rare cancers:
Knowledge database Establishing registrY Asia

National Cancer Center Hospital JAPAN

AMED
国立研究開発法人 日本医療研究開発機構
Japan Agency for Medical Research and Development

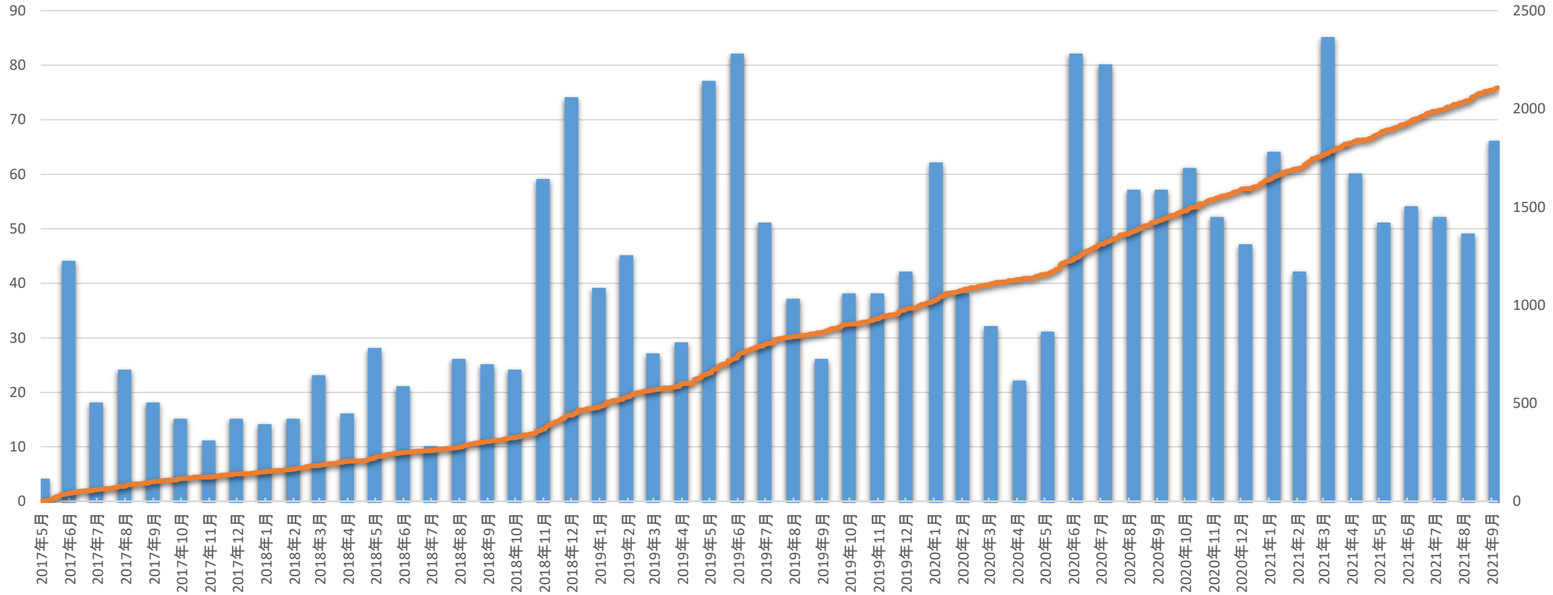
国立がん研究センター
中央病院
National Cancer Center Hospital

ASIAN CLINICAL TRIALS NETWORK
FOR CANCERS PROJECT

MASTER KEY Project (in Japan)

The MK project was started as a joint industry-academia platform to develop treatment for rare cancers (2017)

More than 2000 cases are registered as of November 2021



[Project outline]

Registry research

Biomarkers (genetic abnormalities, protein expression, etc.)

Patients with rare cancers

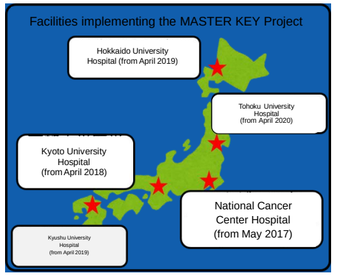
Explanation/consent; Patient registration

Investigate biomarker information

Multiple clinical trials
Participation in clinical trials based on biomarkers

Biomarker A	Biomarker B	....	No biomarkers
Pharmaceutical A Physician-led clinical trial	Pharmaceutical B Corporate treatment	....	Pharmaceutical X Physician-led clinical trial / Pharmaceutical Y Corporate treatment

Follow-up survey of all patients for database construction



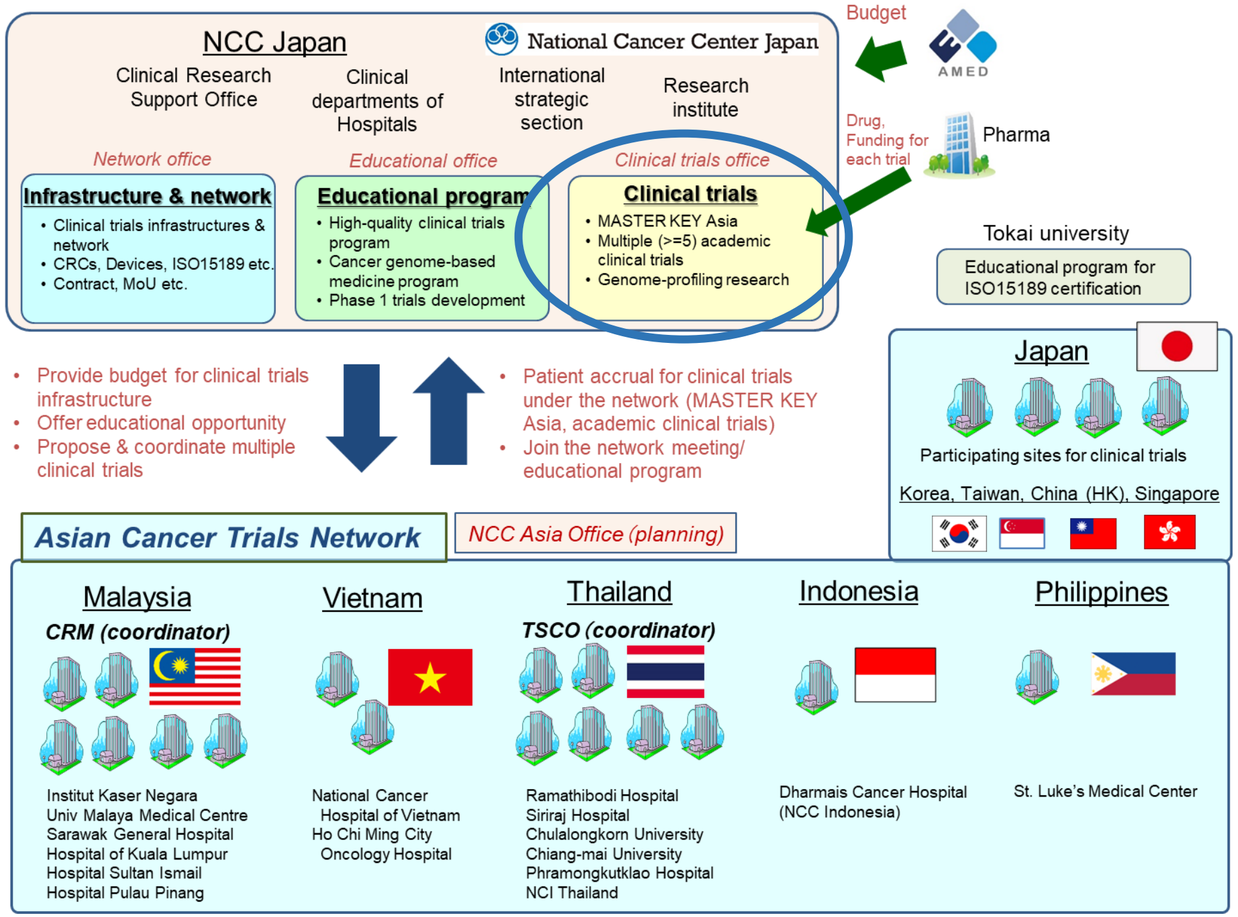
Names of cooperating companies 2021
Astellas Pharma Inc.
Eisai Co., Ltd
Otsuka Pharmaceutical Co., Ltd.
Ono Pharmaceutical Co., Ltd.
Daiichi Sankyo Co., Ltd.
Taiho Pharmaceutical Co., Ltd.
Takeda Pharmaceutical Company Limited
Chugai Pharmaceutical Co., Ltd.
Boehringer Ingelheim (Japan)
Novartis International AG
Pfizer R&D Inc.
Bristol Myers Squibb

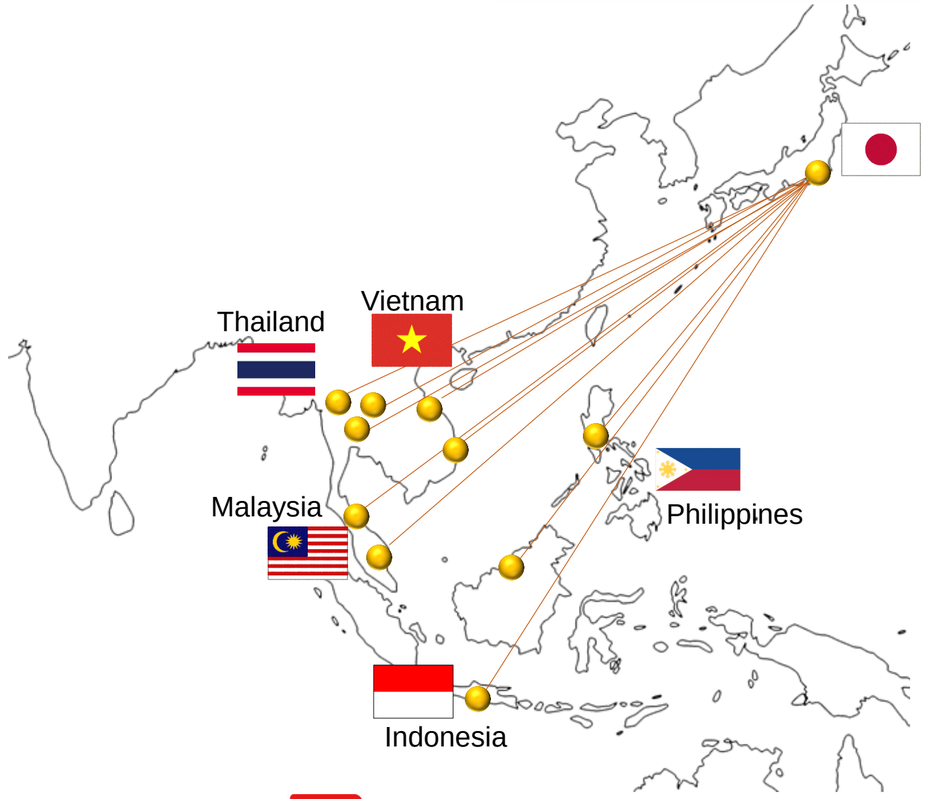
Marker Assisted Selective ThErapy in Rare cancers: Knowledge database Establishing registrY Project

Expansion of the MASTER KEY Project to Asia

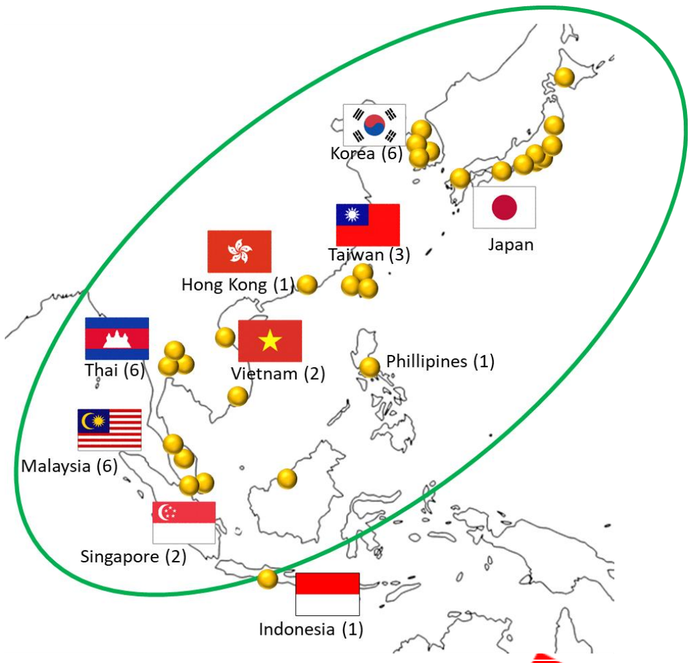
Part of the ATLAS project (Asian clinical TriaLs network for cAncerS)

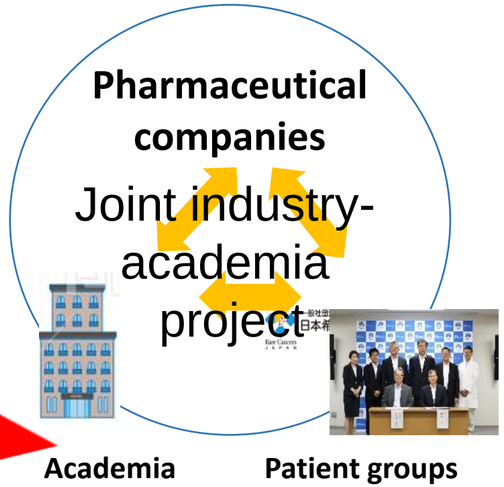
Early-phase drug development network in rapidly growing ASEAN countries





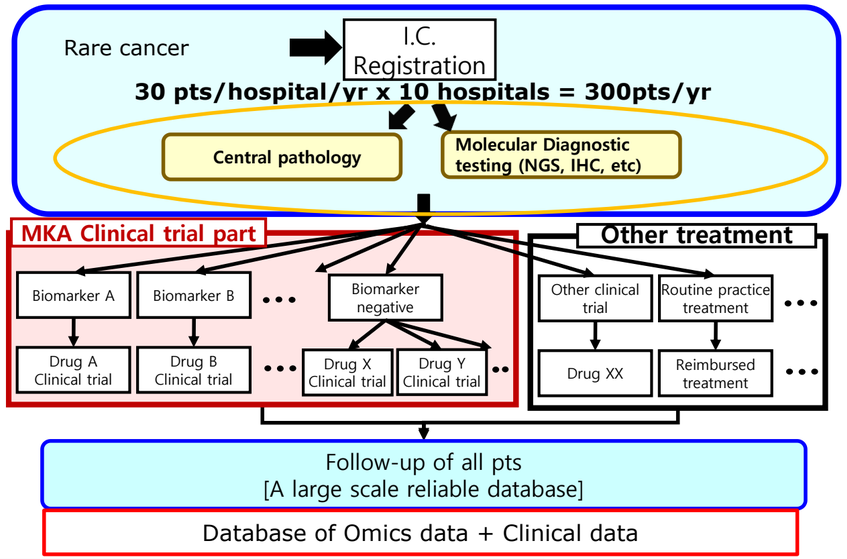
Perspectives Across Asia





Construction of a large-scale database that contains genomic information on rare cancers

- Clarify the characteristics of **rare and common types of cancers in Asia**
- Build historical data
- Register clinical trials based on biomarkers



Implementation of international collaborative investigator-initiated clinical trials aiming for regulatory approval of treatment for rare cancers

- Implement clinical trials based on biomarker status rather than cancer type
- Expand the network focusing on countries in Asia with established health systems and rapid patient registration

MASTER KEY ASIA

Participation of 10 facilities in ASEAN countries

Ramathibodi Hospital

Ho Chi Minh City Oncology Hospital
National Cancer Institute

St Luke’s Medical Center

Sarawak general Hospital
Hospital Sultan Ismail
Hospital Kuala Lumpur
National Cancer Institute
Hospital Pulau Pinang
University Malaya Medical Center
(Planning to participate in the future)

Dharmais Hospital

MASTER KEY ASIA

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

Overall Flow

Send specimens

Input registry data

Diagnostic pathology, gene panel testing

Build biobank

medidata

Send specimens

National Cancer Center Japan

Vietnam

Thailand

Malaysia

Philippines

Indonesia

National Cancer Center Japan

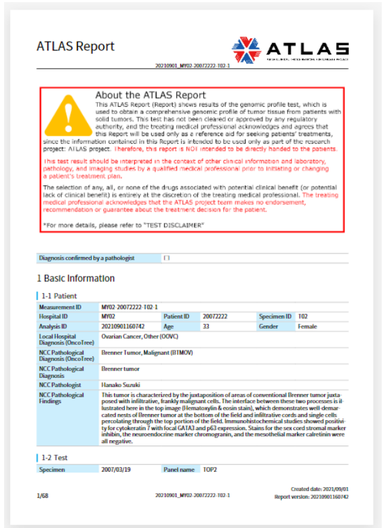
Diagnostic pathology review
Quality control

KONICA MINOLTA NGS analysis

MKI 三井情報株式会社 Report preparation

box

ATLAS Report



Recruit to clinical trials

Result feedback

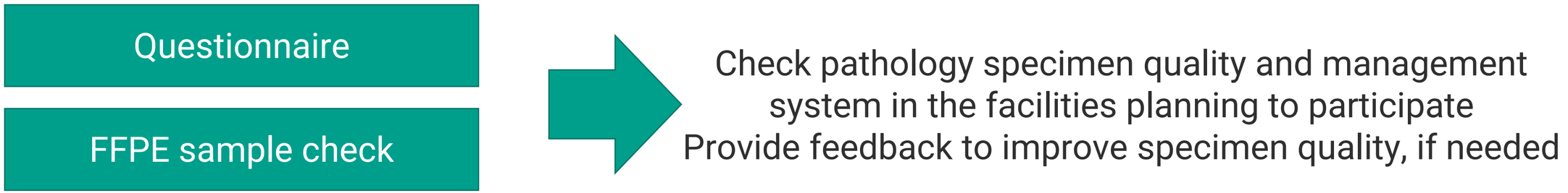
MASTER KEY ASIA

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

Work Implemented by the NCCH Department of Diagnostic Pathology in MASTER KEY ASIA

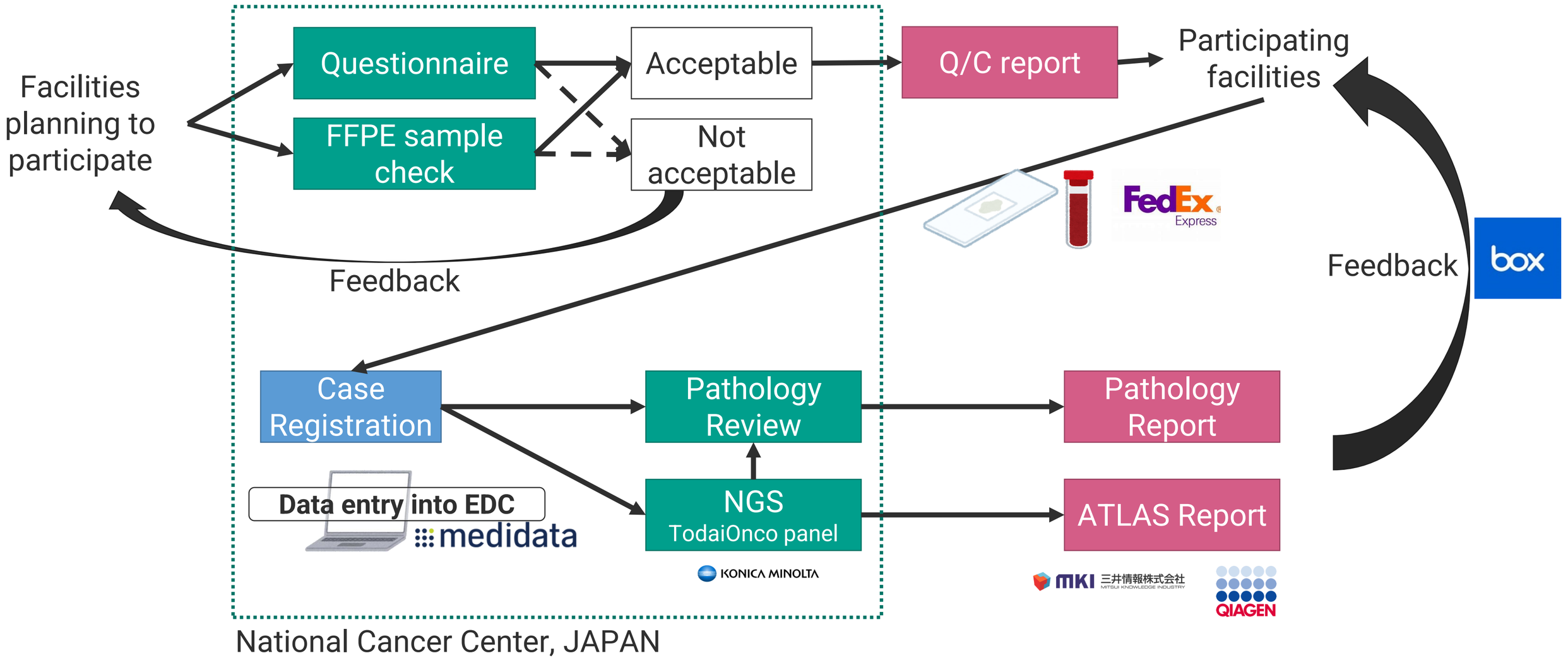
- Before registration of participation: Check pre-analytical factors of pathology specimens in the facilities planning to participate

    1. Check the facility's responses to a questionnaire on their quality control status and pathology specimen preparation process
    2. Have the facility send actual FFPE samples; check IHC and nucleic acid quality



- After registration of participation: Perform pathology reviews of individual cases (also integrate NGS results)

Overall Flow



Expected Results From This Project

- Diagnose rare cancers based on morphological characteristics or immunohistochemical staining or obtain new evidence to support the diagnostic pathology performed in each facility through review by experts who specialize in the pathology of certain organs.
- Definitive diagnosis based on morphology is difficult, but diagnosis can be achieved through measures such as identification of fusion genes.

  (Recently, many disease units have been established on the basis of molecular biological abnormalities, and this trend is strong in rare cancers)
- Even if a definitive histopathological diagnosis is not achieved, molecular abnormalities that are therapeutic targets can be identified with NGS analysis.
- Establish a database linking the diagnostic pathology of rare cancers with genomic abnormalities in Asia.
- Searching for biomarkers is essential for future clinical trials, and ensuring the quality of FFPE samples forms the basis of these clinical trials.

Checking Pre-analytical Factors of Pathology Specimens

Why is this necessary?

- MASTER KEY ASIA includes rare cancers that are difficult to diagnose; therefore, ancillary tests, such as immunohistochemical staining, *in situ* hybridization, and NGS, may be required.

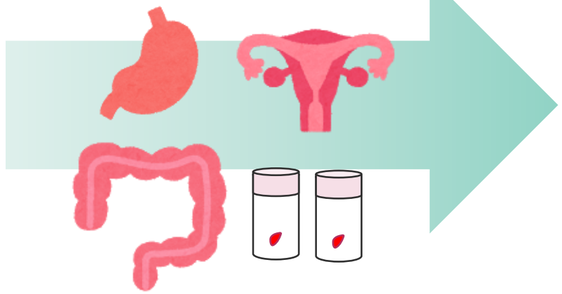
- Pre-analytical factors (time to fixation, type of fixing agent, composition, fixation time, etc.) significantly affect the final result in ancillary tests; therefore, various guidelines have been proposed worldwide to maintain the accuracy of these tests.

Diagnostic Pathology In Clinical Practice

Pre-analytical variables

Doctors from each clinical department

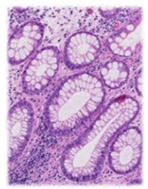
Specimens
Resected organs/ biopsy tissue



Department of Diagnostic Pathology

Sample preparation
(Pathologist/clinical laboratory technician)

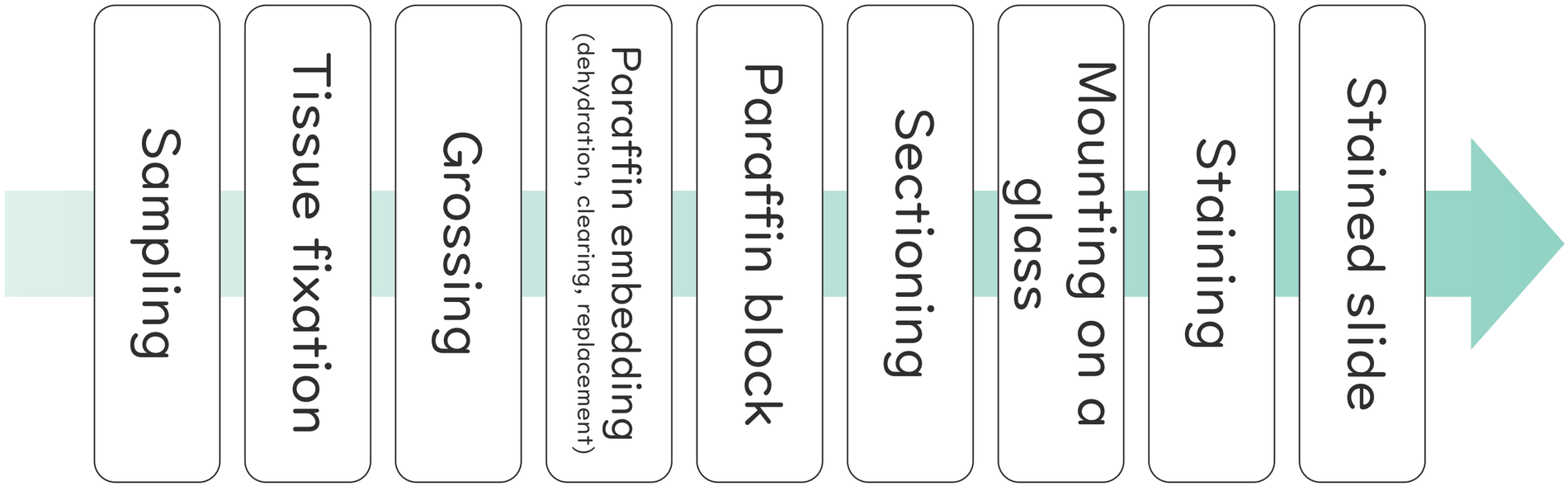
Sample observation and diagnostic pathology (pathologist)



Diagnostic pathology report

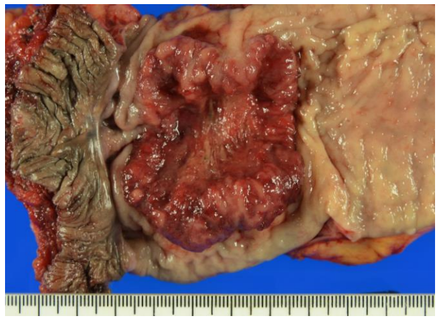
Decide treatment strategy

The sample preparation process is directly linked to the quality of diagnostic pathology



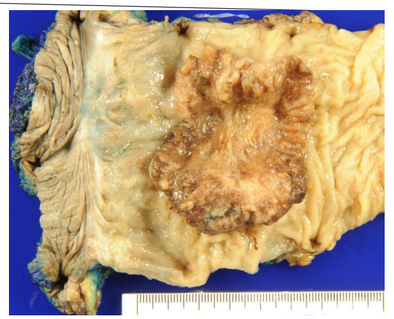
- The sample preparation process affects the quality of diagnostic pathology.
- An observable quality sample containing a suitable part of the specimen is required for a correct diagnosis.
- Process control is required for molecular pathology searches.

Sampling → Tissue fixation → Grossing → Paraffin embedding (dehydration, clearing, replacement) → Paraffin block → Sectioning → Mounting on a glass → Staining → Stained slide



Collect fresh tissue as needed

Time to starting tissue fixation
Cold ischemic time

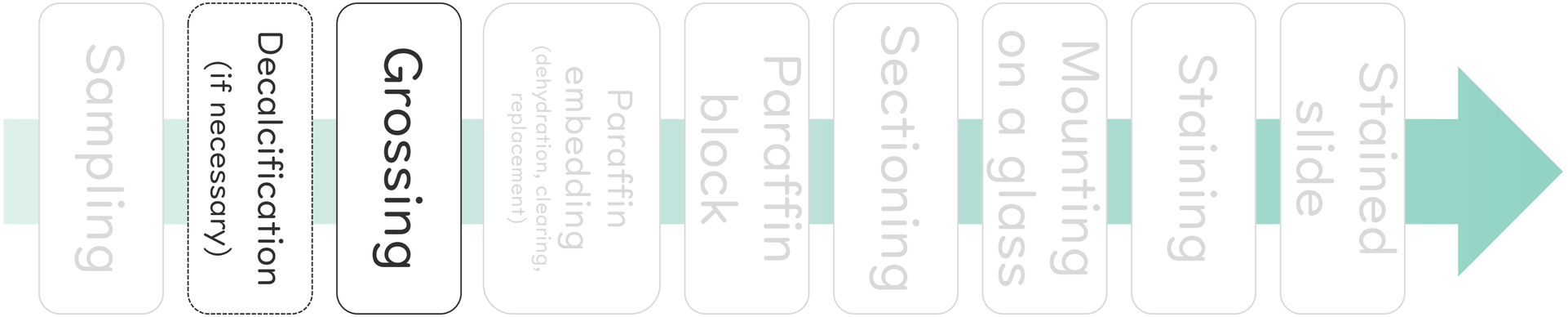


Not performing prompt and proper tissue fixation damages the tissue morphology and the nucleic acids and proteins in the tissue, making pathological diagnosis and molecular pathology search difficult.

- Do not leave the resected organ at room temperature for more than 30 minutes.
- If fixation cannot be performed immediately, store the organ in a refrigerator (4℃), and fix within about 3 hours.
- Ensure sampling for genomic research does not interfere with diagnostic pathology.

- 10% neutral buffered formalin is recommended as a fixative from the perspective of protein antigenicity and maintaining the quality of nucleic acid (this is also a requirement in international clinical trials).
- 6 to 72 hours is recommended as the tissue fixation time (most genetic tests are within 48 hours)
- Formalin penetration rate is approximately 1 mm/hour

The Japanese Society of Pathology Guidelines on the handling of pathological tissue samples for genomic research: Standard operating procedures based on empirical analyses.
Kanai et al. Pathology International 2018; 68: 63−90

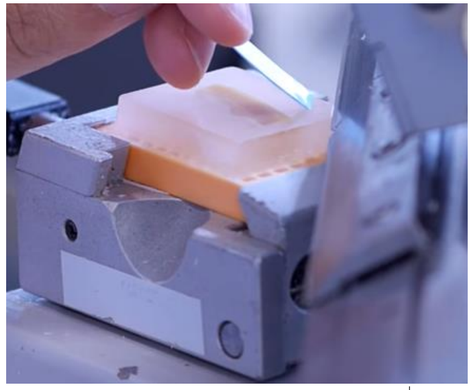


- Grossing involves selecting the site for microscopic observation based on macroscopic observations, bearing in mind the pathological findings required for treatment.
- When sending a pathology specimen based on a referral from another hospital, it is advisable to also attach a gross description/section code.
- Hard tissue is treated with decalcification, but acid decalcification should be avoided (EDTA decalcification is recommended).

Sampling → Tissue fixation → Grossing → Paraffin embedding (dehydration, clearing, replacement) → Paraffin block → Sectioning → Mounting on a glass → Staining → Stained slide

FFPE block

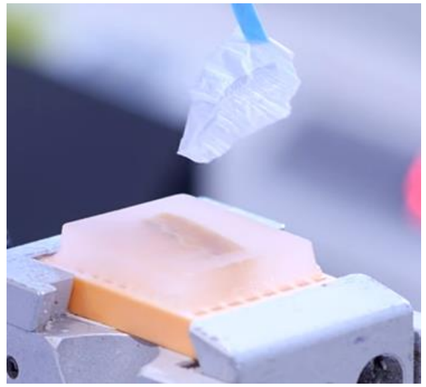
Formalin-fixed paraffin embedded



Sectioning with a microtome

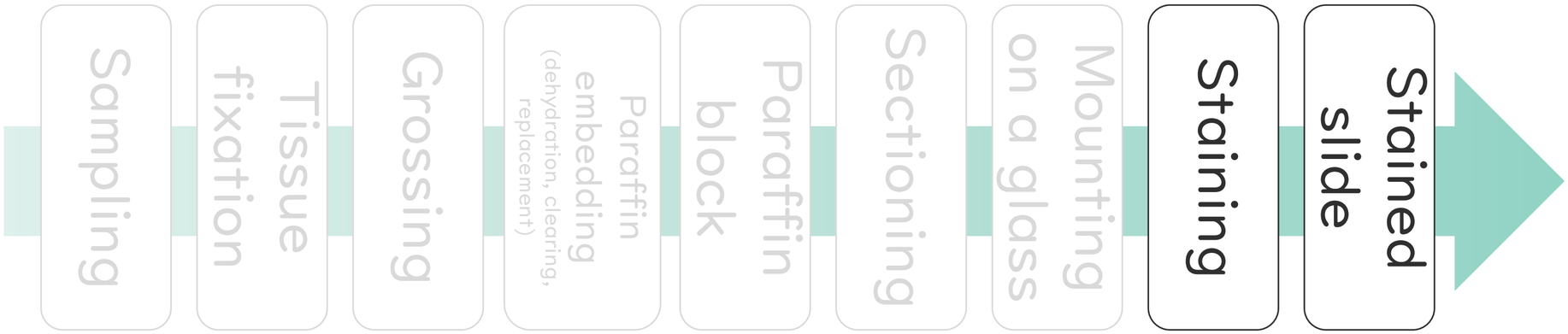
- Thickness approximately 4μm

*Different slides have different sectioning “surfaces” even with the same block. For example, new findings may be obtained from deeper sections because small tumor tissue may not appear in superficial sections.

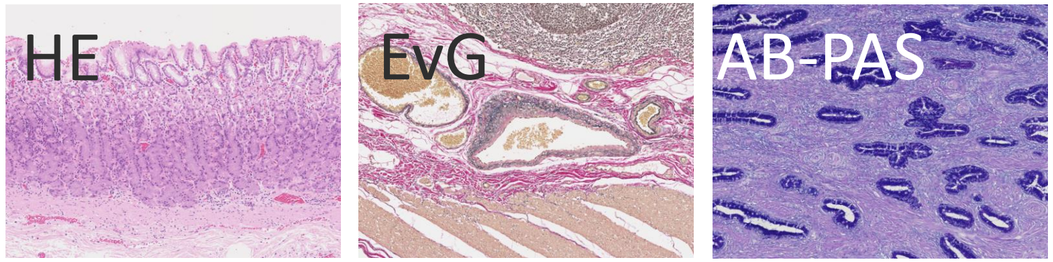


Affix to glass

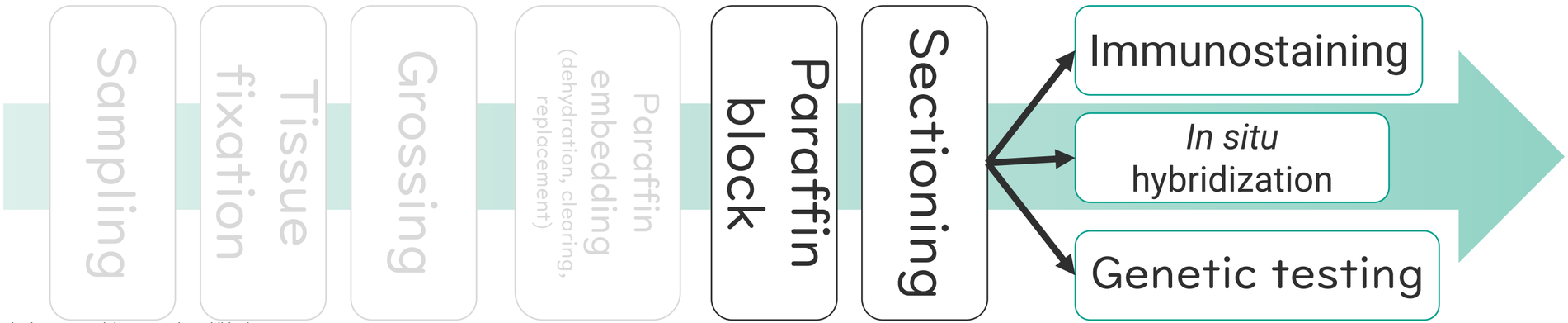
- Unstained sample
- Coated slide
  (Treated to prevent tissue separation)
  → Required for immunostaining and ISH



- Unstained sample → H&E staining (basic), special staining (chemically enhances tissue components)



- If an FFPE block is stored, it can be provided for various molecular pathological searches.
- However, long-term storage causes degradation of nucleic acids. Reports indicate that it is preferable to use FFPE blocks within 3 years after preparation to ensure highly reliable genetic testing.



Management of the preanalytical factors of pathology specimens is essential for tests examining protein, DNA, and/or RNA

Each guideline has recommendations for Cold ischemia time, Fixation, Storage, and other processes

Table 1. Preanalytical Recommendations for Tissue for Molecular Analysis From National and International Authoritative Sources

	Organization				
	CAP-ASCO 2014	CLSI ILA28-A2 2011	CLSI MM13 2005	NCI-BBRB 2016	CAP General Surg Path 2015
Biomolecule/biomarker	Breast biomarkers	Proteins: IHC	DNA, RNA, protein	Molecular analysis	General surgical pathology
Preanalytical parameter					
Cold ischemia time, min	60 min or less	As short as possible (min)	60 min or less	As short as possible, optimally less than 20 min but no more than 1 h	<12 h
Handling and processing temperature	—	—	—	—	Room temperature (22°C–25°C)
Total time in formalin, h	6–72 h	Greater than 8 h; no longer than 12–36 h	Not to exceed 72 h (for nucleic acids)	—	A minimum of 6 h; a maximum of 48 h (actual time should be recorded)
Type of fixative	10% Neutral phosphate-buffered formalin	10% Neutral phosphate-buffered formalin	10% Neutral phosphate-buffered formalin	—	10% Neutral buffered formalin
Volume ratio of formalin to tissue	—	>10:1	—	—	15–20:1
Type of paraffin (low melt <60°C versus high melt ≥60°C)	—	55°C–58°C	—	—	—
Quality of tissue-processing fluids	—	Changed in a timely fashion	Per manufacturer's specifications	—	—
Thickness of tissue section into cassette	—	2 mm or less	—	—	4 mm
Temperature of block storage	—	—	—	Temperatures below 80°F (27°C)	—

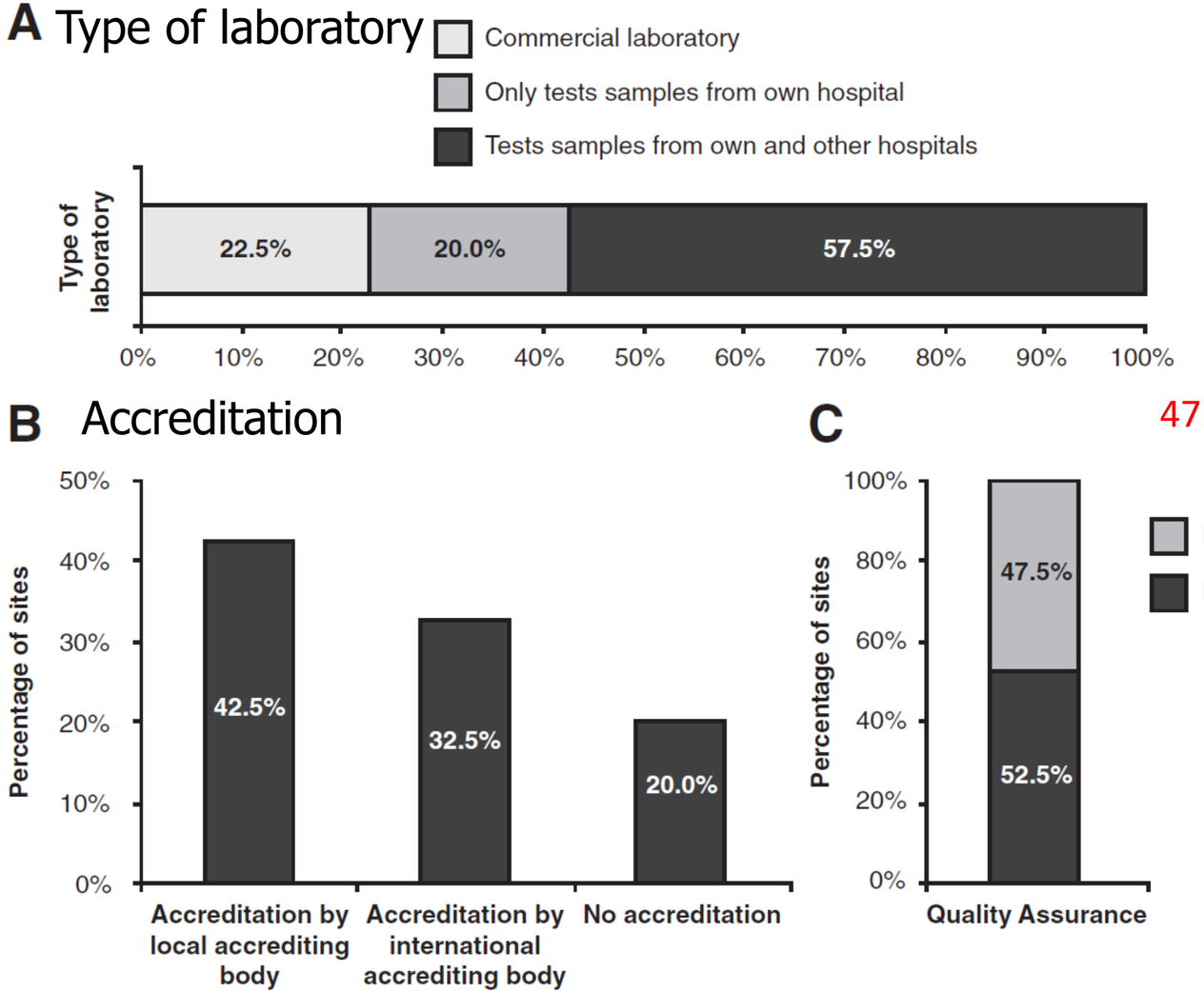
Table 1. Extended

	Organization				
	ISO/TC-212 2016	ISO/TC-212 2016	CEN/TC 140 2015	CEN/TC 140 2015	CEN/TC 140 2015
Biomolecule/biomarker	Isolated RNA	Isolated DNA	Isolated protein	Isolated RNA	Isolated DNA
Preanalytical parameter					
Cold ischemia time, min	60 min or less	Avoid cold ischemia when possible. Document actual cold ischemia time and warm ischemia time	As short as possible (document actual cold ischemia time and warm ischemia time)	Avoid cold ischemia when possible (directly into standard buffered formalin). If unavoidable: as short as possible. Documentation required	Avoid cold ischemia when possible (directly into standard buffered formalin). If unavoidable: as short as possible. Documentation required
Handling and processing temperature	—	Room temperature (18°C–25°C)	Room temperature (18°C–25°C)	Room temperature (18°C–25°C)	Room temperature (18°C–25°C)
Total time in formalin, h	<72 h (old) 24–36 h (revised 2016)	12–24 h	12–24 h	Less than 24 h, eg, 12–24 h for tissue thickness of 5 mm. Documentation required	12–24 h for tissue thickness of 5 mm. Optimal duration can vary depending on tissue type and size. Documentation required
Type of fixative	10% Neutral phosphate-buffered formalin	10% Neutral buffered formalin (pH and concentration checked daily before use and with each new batch) [type of buffer not specified]	10% Neutral buffered formalin (pH and concentration checked regularly) [type of buffer not specified]	10% Neutral formalin solution pH 6.8–7.2 (type of buffer not specified)	10% Neutral formalin solution pH 6.8–7.2 (type of buffer not specified)
Volume ratio of formalin to tissue	—	15–20:1	At least 10:1	4:1 at least; optimal 10:1	4:1 at least; optimal 10:1
Type of paraffin (low melt <60°C versus high melt ≥60°C)	—	Low melt	Low melt	Low melt	Low melt
Quality of tissue-processing fluids	Well maintained	Per manufacturer's specifications	Per manufacturer's specifications	Per manufacturer's specifications	Per manufacturer's specifications
Thickness of tissue section into cassette	4–5 mm	5 mm	5 mm	Max. 5 mm	Max. 5 mm
Temperature of block storage	—	Room temperature (18°C–25°C) or lower	Room temperature (18°C–25°C) or lower	Room temperature (18°C–25°C) or lower	Room temperature (18°C–25°C) or lower

Abbreviations: ASCO, American Society of Clinical Oncology; BBRB, Biorepositories and Biospecimen Research Branch; CAP, College of American Pathologists; CEN/TC, European Committee for Standardization/Technical Committee; CLSI, Clinical & Laboratory Standards Institute; IHC, immunohistochemistry; ISO/TC, International Standards Organization/Technical Committee; NCI, National Cancer Institute; Surg Path, Surgical Pathology.

Compton et al. Arch Pathol Lab Med. 2019;143: 1346–1363

Quality assurance in laboratories is not always widespread in Asian countries



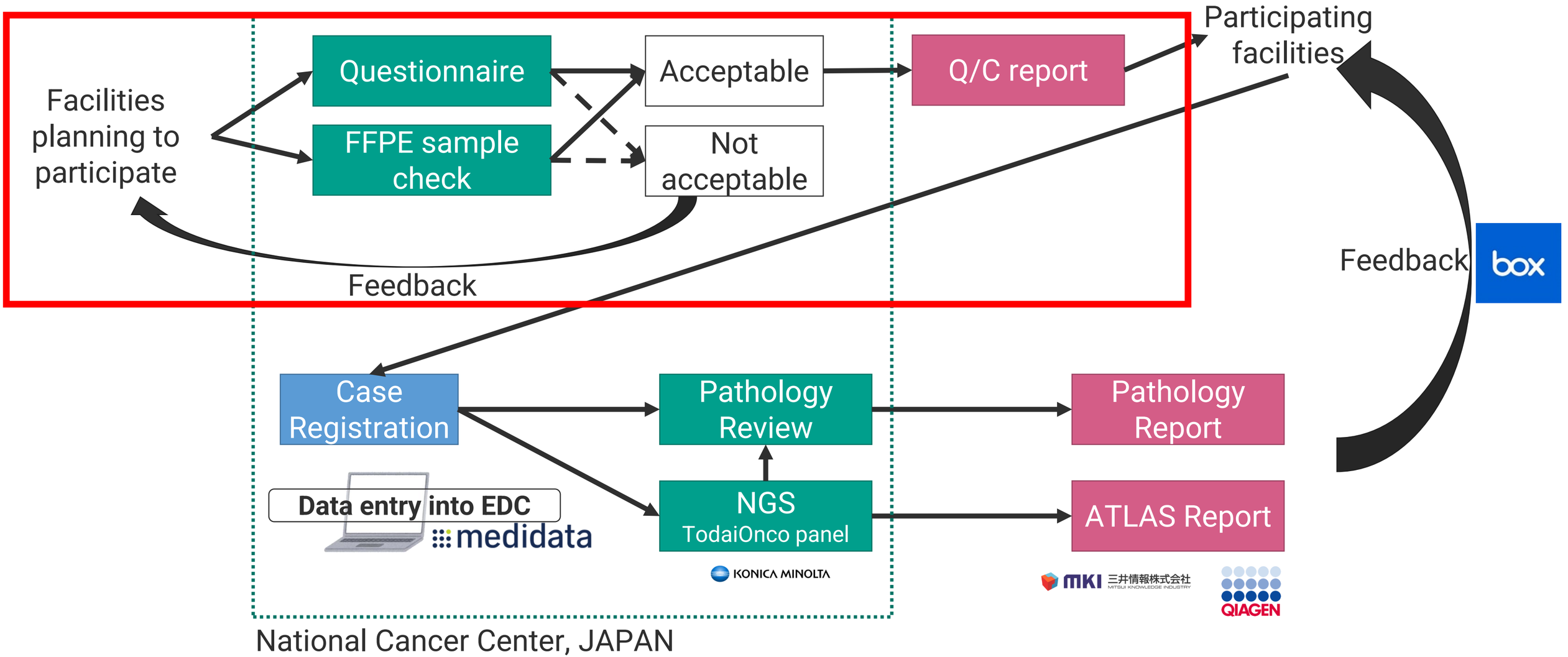
A retrospective survey of records from NSCLC patients tested for *EGFR* mutations during 2011 was conducted in 11 Asian-Pacific countries at 40 sites that routinely performed *EGFR* mutation testing during that period.

47.5% were not members of a Quality Assurance Scheme.

FIGURE 3. Information about EGFR mutation testing sites that participated in the survey. A, Type of laboratory; B, accreditation; and C, participation in QA schemes amongst participating laboratories. EGFR, epidermal growth factor receptor; QA, quality assurance

Yatabe Y, Kerr KM, Utomo A, et al. EGFR mutation testing practices within the Asia Pacific region: results of a multicenter diagnostic survey. *J Thorac Oncol* 2015;10: 438-445.

Overall Flow



Questionnaire on the status of quality control in the facility and the pathology specimen preparation process

- Part I. Facility laboratory certification, quality control status, actual implementation status of companion diagnostics, and results of submission of pathology samples to international collaborative clinical trials.

- Part II. Detailed process of sample preparation : Time to tissue fixation, type and composition of fixation material, regular management status, tissue fixation time, paraffin types, storage, and transportation environment, etc.

Questionnaire Sheet

Laboratory quality and management of preanalytical variables of FFPE specimens

Part I. Questions about laboratory quality management

① Is your laboratory accredited, certified, or similar ? (multiple answers possible)

☐ ISO15189, ☐ CLIA(Clinical Laboratory Improvement Amendments),
☐ National Standard: please specify ( ), ☐ Ongoing accreditation / certification
☐ No accreditation / certification

② Has your laboratory ever submitted FFPE specimens to an international clinical trial for immunohistochemistry or sequencing?

☐ Yes / If yes, please specify, ☐ No

Questionnaire Sheet

Laboratory quality and management of preanalytical variables of FFPE specimens

③ Does your laboratory perform IHC as companion diagnostics? (for e.g., uses *in vivo* diagnostics kit of HER2 immunohistochemistry for breast cancer)

☐ Yes / If yes, please specify (　　), ☐ No

④ If yes, could you kindly inform us of the annual number of HER2 immunostainings performed for breast cancer and the percentage of cases with a score of 3+?

☐ the annual number of HER2 IHC for breast cancer: _____cases,

☐ the percentage of cases with a score 3+: _____%

⑤ Does your laboratory participate in an external quality assurance (EQA) program for immunohistochemistry (e.g., CAP survey, UK NEQAS, NordiQC, or another EQA program)?

☐ Yes / If yes, please specify, ☐ No

Part II. Questions about management of pre-analytical variables of FFPE specimens

- These questions are based on the published recommendations by the CAP Personalized Healthcare Committee established by the Pre-analytics for Precision Medicine Project Team (Arch Pathol Lab Med. 2019;143: 1346–1363)

① Questions about cold ischemia time or time to fixation, defined as the length of time between removal of the biospecimen from the patient and stabilization of the biospecimen in formalin (i.e., the biological activity in the tissue is stopped by fixing).

- What is the approximate range of cold ischemia time for the specimens at your institution?

  ☐ 0-1 hr ☐ 1-3 hr ☐ 1-6 hr ☐ 1-12 hr

- What is the approximate median of cold ischemia time for the specimens at your institution?

  ☐ 1 hr ☐ 3 hr ☐ 6 hr ☐ 12 hr

- Is the cold ischemia time of the specimens recorded so that it can be traced? ☐ Yes / ☐ No

- Before fixation, are the specimens stored in a refrigerator (4 ℃ / 40 ℉) or at room temperature (25 ℃ / 77 ℉)?

  ☐ refrigerator (4 ℃ / 40 ℉) / ☐ room temperature (25 ℃ / 77 ℉) / ☐ room temperature (>25 ℃ / 77 ℉)

Part II. Questions about management of pre-analytical variables of FFPE specimens

② Questions about fixatives

- Type of fixative: ☐ 10％ neutral phosphate-buffered formalin or ☐ Other ( )
- Is the pH of formalin checked on a regular basis? ☐Yes / ☐No
- Is the formalin replaced on a regular basis? ☐Yes / ☐No
- Decalcification solution: ☐EDTA solution / ☐other acidic solution ( )

③ Questions about fixation time

- Fixation time for biopsy specimens (range, hours):
- Fixation time for surgically resected specimens (range, hours):
- Temperature of fixation room: ☐~25 ℃ / 77 ℉ ☐>25 ℃ / 77 ℉

④ What is the approximate volume ratio of formalin to a specimen in your laboratory?

☐1: 1 ☐ 2-4: 1 ☐4-10: 1 ☐>10: 1

⑤ Is a large specimen appropriately cut before fixation? ☐ Yes / ☐ No

Part II. Questions about management of pre-analytical variables of FFPE specimens

⑥ Has tissue processor maintenance been routinely performed according to the manufacturer's instruction?

☐ Yes / ☐No

⑦ What type of paraffin is used for embedding the tissue in your laboratory?

☐ Pure Low-Melt Paraffin (Melts at ,<60 ℃) ☐ Other

⑧ Are all the paraffin blocks stored in dry, pest-free conditions at room temperature (Defined as around 25 ℃)?

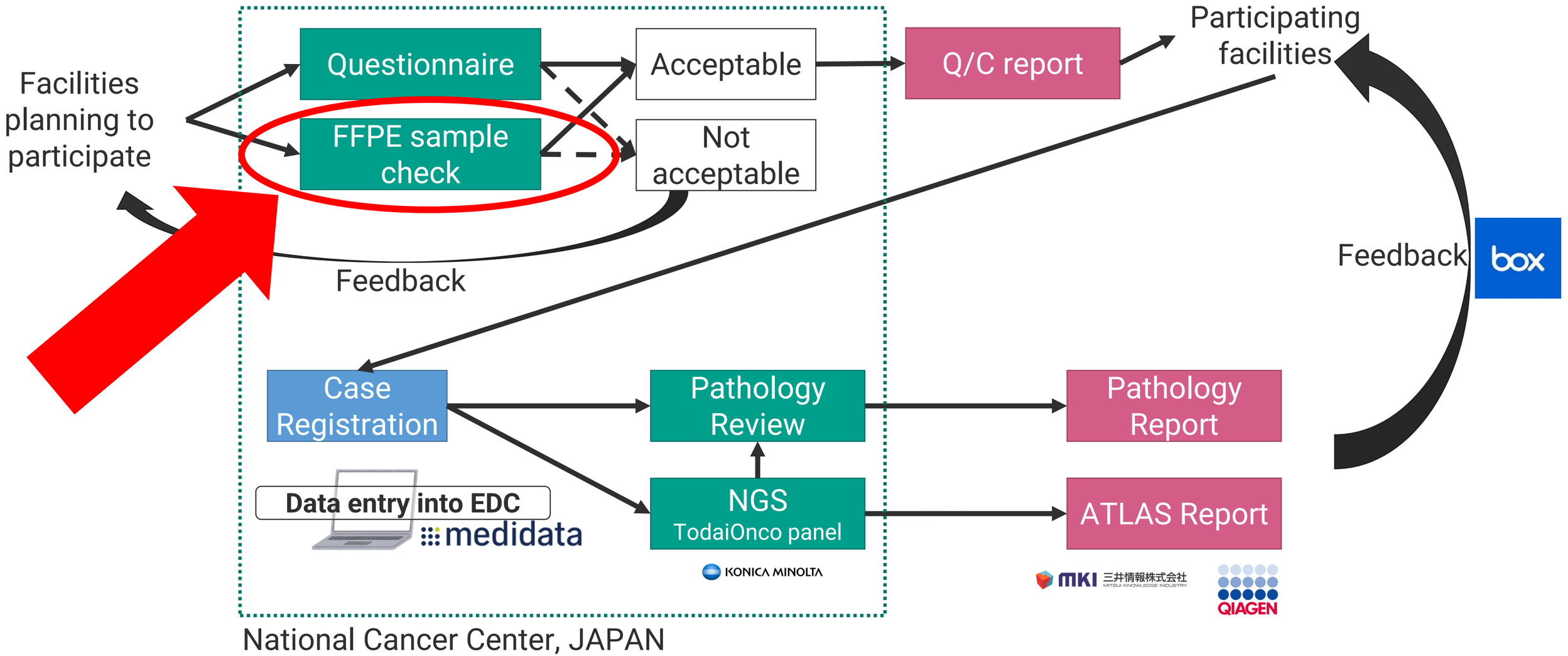
☐Yes / ☐No (_____)

⑨ Please tell us how long it will take from the time the slides are prepared to the time they are shipped, as well as the temperature of the location where they will be stored until they are shipped.

- Time to shipping of unstained slides: ☐ 1 day ☐<1 week ☐ 1-3 weeks ☐ 3 weeks<
- Temperature of stored unstained slides: ☐ 4 ℃ ☐ 4-25 ℃ ☐>25 ℃

If there are factors that could be improved among the responses, feedback is provided to the facilities planning to participate

Overall Flow



Sample Requisition Form

Individual samples should be submitted with the following pre-analytical history and sample information.

- Sample ID: _____
- Date sampled: _____
- Organ/Site of the specimen: _____
- Sample preanalytical history

1. Time to fixation: ( ) hours
2. Fixative type: 10% NBF (neutral buffered formalin) or others ( )
3. Fixation time: ( ) hours
4. Fixation temperature: ~25℃ or more than 25℃ (℃)
5. Decalcification: Yes / No

Checking the quality of IHC and nucleic acids in FFPE samples

- The quality control status of specimens from facilities planning to participate is checked through the questionnaire. Thereafter, the quality of the FFPE samples is checked, and it is confirmed whether these samples can be used for the immunostaining and sequencing required for this project.
- Each of the facilities planning to participate is requested to submit the following samples, which are then tested in NCC.

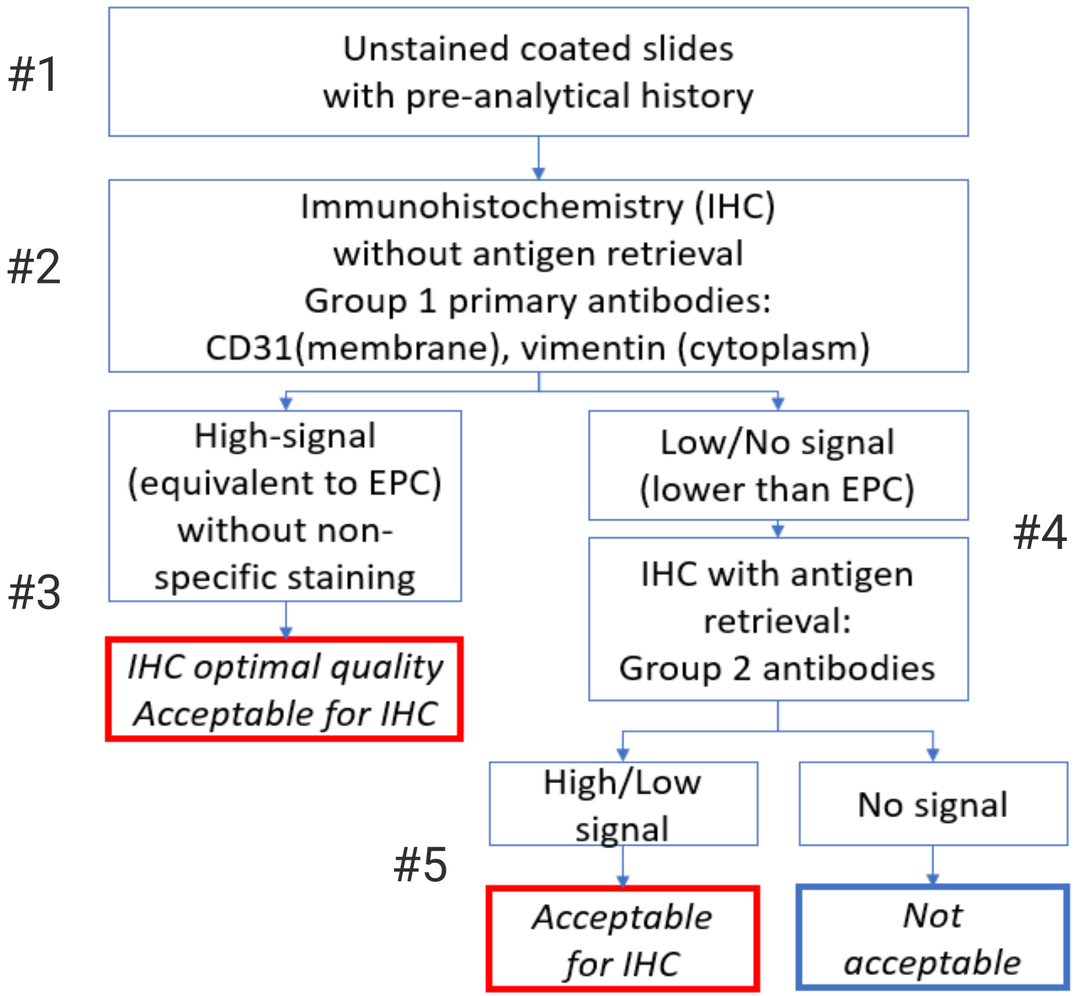
- Five-micrometer-thick unstained sections, fixed with 10% buffered formalin for 6 to 78 hours
- One HE-stained section and 20-40 unstained sections, according to the tissue size
  - ➢10 sections of 4-5 um thick unstained sections on charged slides for IHC check
  - ➢10-30 unstained sections on uncharged slides for molecular panel testing*

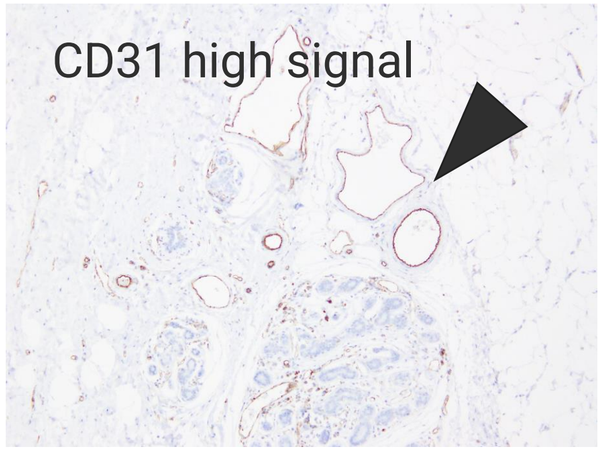
•Note: Number of sections needed for panel testing depends on tissue size.

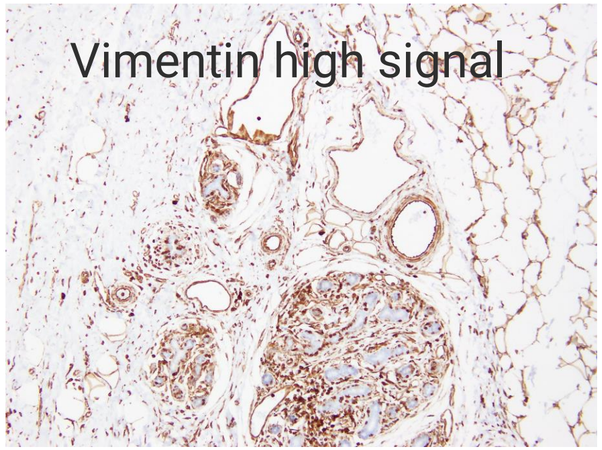
Tissue size (mm2)	Suggested number of 4-5 um thick sections (in case of 10um thick)
4	30 (20)
16	20 (10)
24	10 (5)

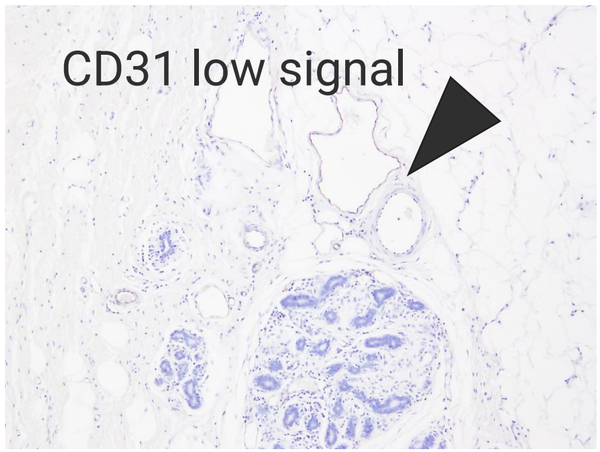
1. **Immunohistochemistry**

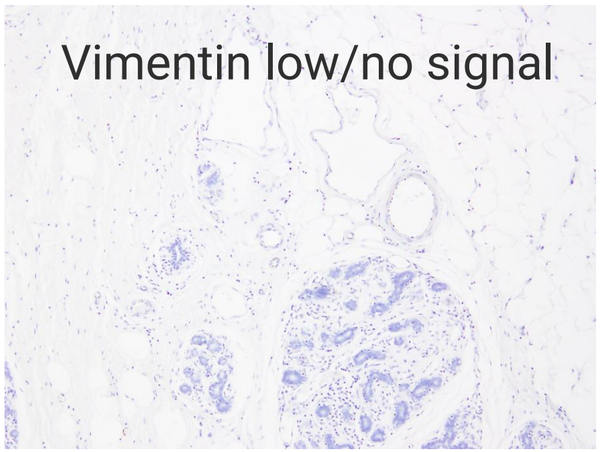
Specimen integrity for immunohistochemistry will be confirmed by the following steps:





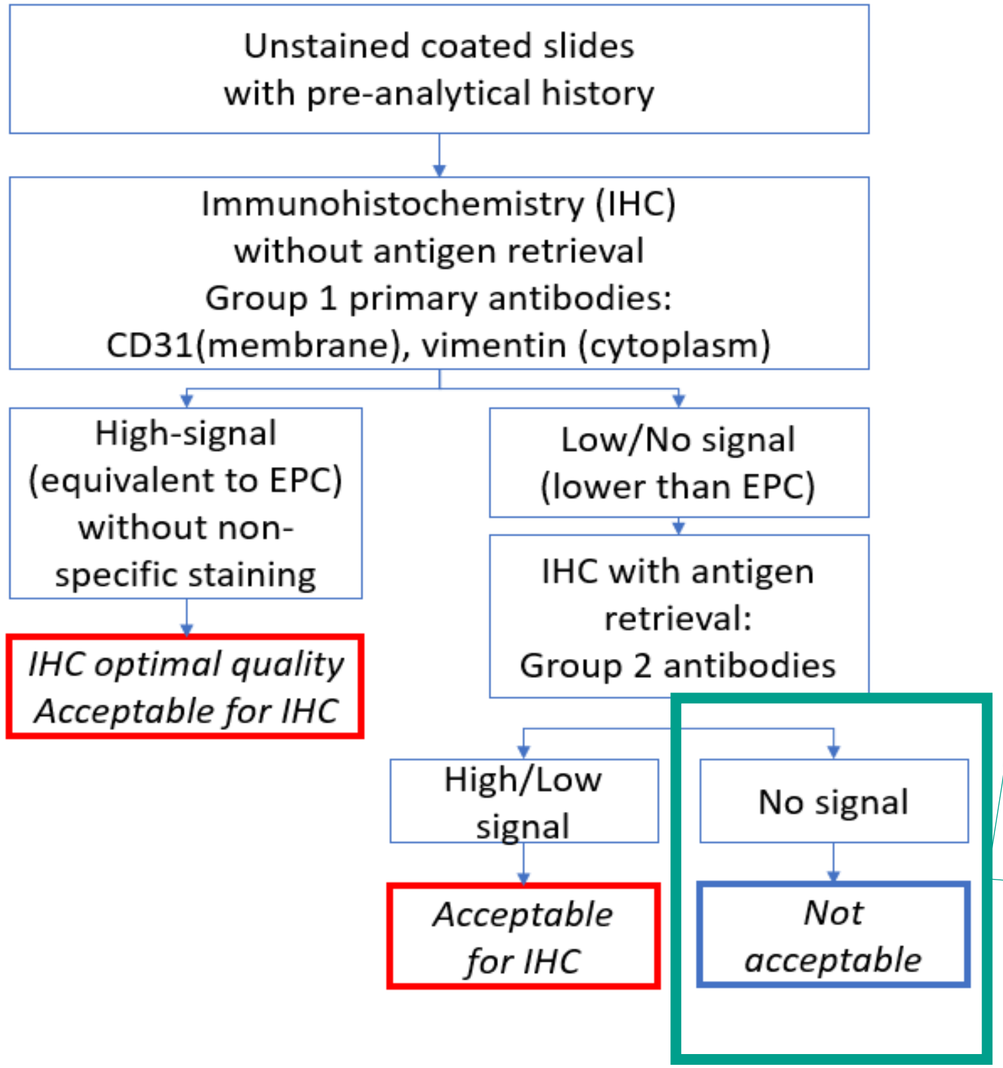






Esteva-Socias et al . Journal of Translational Medicine (2019) 17: 370.

In case of repeated staining failure…



A retest will be planned after optimization of pre-analytical variables.

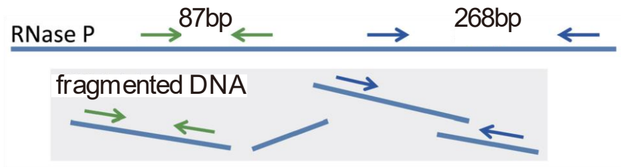
If the specimen passes the retest, the laboratory can participate in this project.

1. In case of staining failure, if a problem in the pre-analytics cannot be corrected by the submitting laboratory, participation to ATLAS project will be denied.
2. In case of staining failure, if the problem with the pre-analytics cannot be identified, participation to ATLAS will be denied because improvement is impossible.

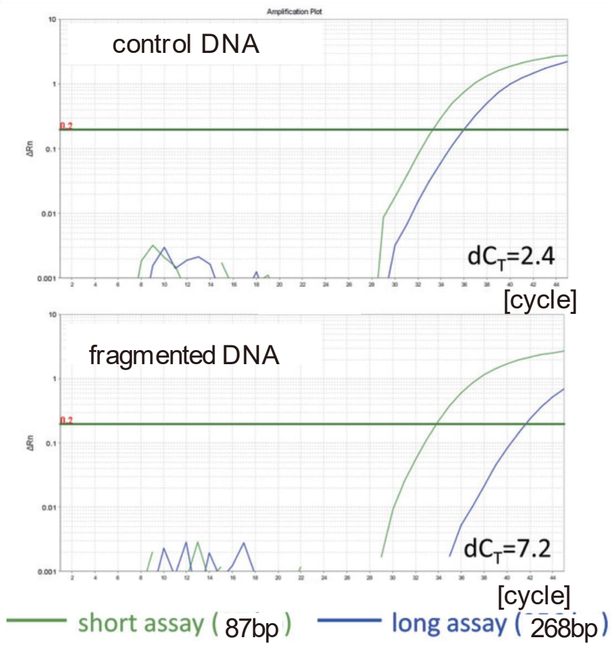
2. DNA and RNA Integrity

Extraction of nucleic acids (DNA and RNA)

- DNA and RNA are extracted simultaneously with a standard procedure using QIAcube according to the manufacture’s instruction.



Fragmented DNA templates show higher CT values than high quality DNA templates especially in analyses using long primer pairs.

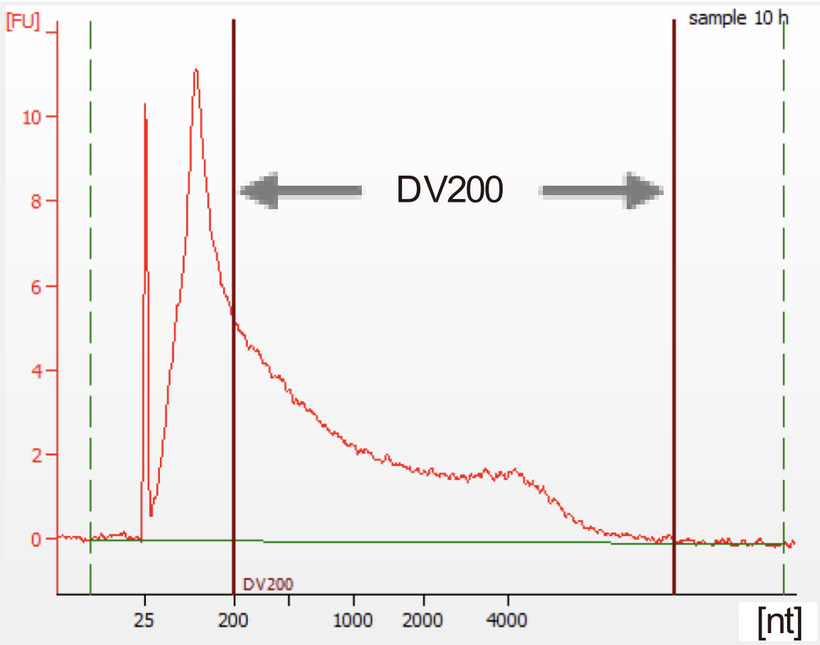


DNA assessment: Amplifiable DNA was quantified using the Thermo Fisher NGS FFPE QC kit, a qPCR-based assay with two sets of primer pairs targeting short (87bp) and long (268bp) regions of the RNaseP gene. The ratio is calculated by "(DNA concentration of qPCR/ DNA concentration by Qubit ssDNA kit) x 100". Short (42bp) and long (123bp) primer pairs used in the Agilent kit.

Sample ID	Δ Ct (Ct long - Ct short)	amplifiable DNA with short primers (%)	amplifiable DNA with long primers (%)	Ref. ΔΔCq (Agilent)
Acceptable value	<8			(<2.4)
Sample	X1	X2	X3	X4

DNA and RNA Integrity

RNA assessment: The DV200 values, which represent the percentage of fragments >200 nucleotides, in RNA samples extracted from FFPE are calculated using the Agilent 2100 Bioanalyzer. Degraded RNA samples show lower DV200 than high quality RNA samples.



Standards

The cut-off values of the quality check in DNA samples should be determined after preliminary analyses.
DV200 > 30% is recommended in RNA samples for further NGS analyses.

Sample ID	DV200 (%)
Acceptable and desirable values	>30% and >40%
Sample	X

“FFPE sample Quality Check Report” is sent to facilities planning to participate

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FFPE sample Quality Check Report

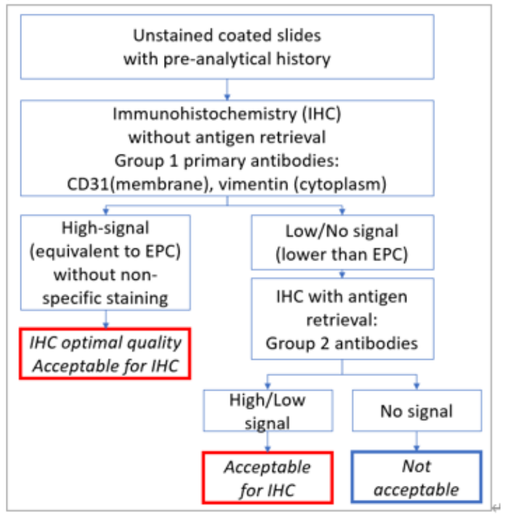
Study ID [redacted]
Hospital: [redacted]
Issued date: [redacted]

Submitted FFPE tissues

1.	Breast

1. Immunohistochemistry

The quality of submitted FFPE samples for immunohistochemical analysis was accessed with the method by the report by Esteva-Socias et al. (J Transl Med 2019;17:370)



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1.1 Results

Step 1	Vimentin w/o AR	CD31 w/o AR	
1	Optimal	Low signal	
Step 2	**Vimentin with AR**	**CD31 with AR**	**Transcription factor**
1	Not performed	Optimal	Not performed

1.2 Assessment

Quality of FFPE specimen for IHC: Acceptable

2. Nucleic acids (DNA and RNA)

DNA and RNA were extracted simultaneously with a standard procedure using Maxwel-FSC-Instrument according to the manufacture’s instruction.

2.1 DNA assessment

Amplifiable DNA was quantified using the Thermo Fisher NGS FFPE QC kit, a qPCR-based assay with 2 sets of primer pairs targeting short (87bp) and long (268bp) regions of RNaseP gene. The ratio was calculated by "(DNA concentration of qPCR/ DNA concentration by Qubit ssDNA kit) x 100". Primer pairs of short (42bp) and long (123bp) were used in Agilent kit.

Results

Sample ID	Δ Ct (Ct long - Ct short)	amplifiable DNA with short primers (%)	amplifiable DNA with long primers (%)	Ref. ΔΔCq (Agilent)
Acceptable value	<8			(<2.4)
1.	3.5	8	3	1.3

2.2 RNA assessment

The DV200 value, the percentage of fragments with more than 200 nucleotides, was calculated using the Agilent 2100 Bioanalyzer.

Results

Sample ID	DV200 (%)
Acceptable and desirable values	>30% and >40%
1.	74

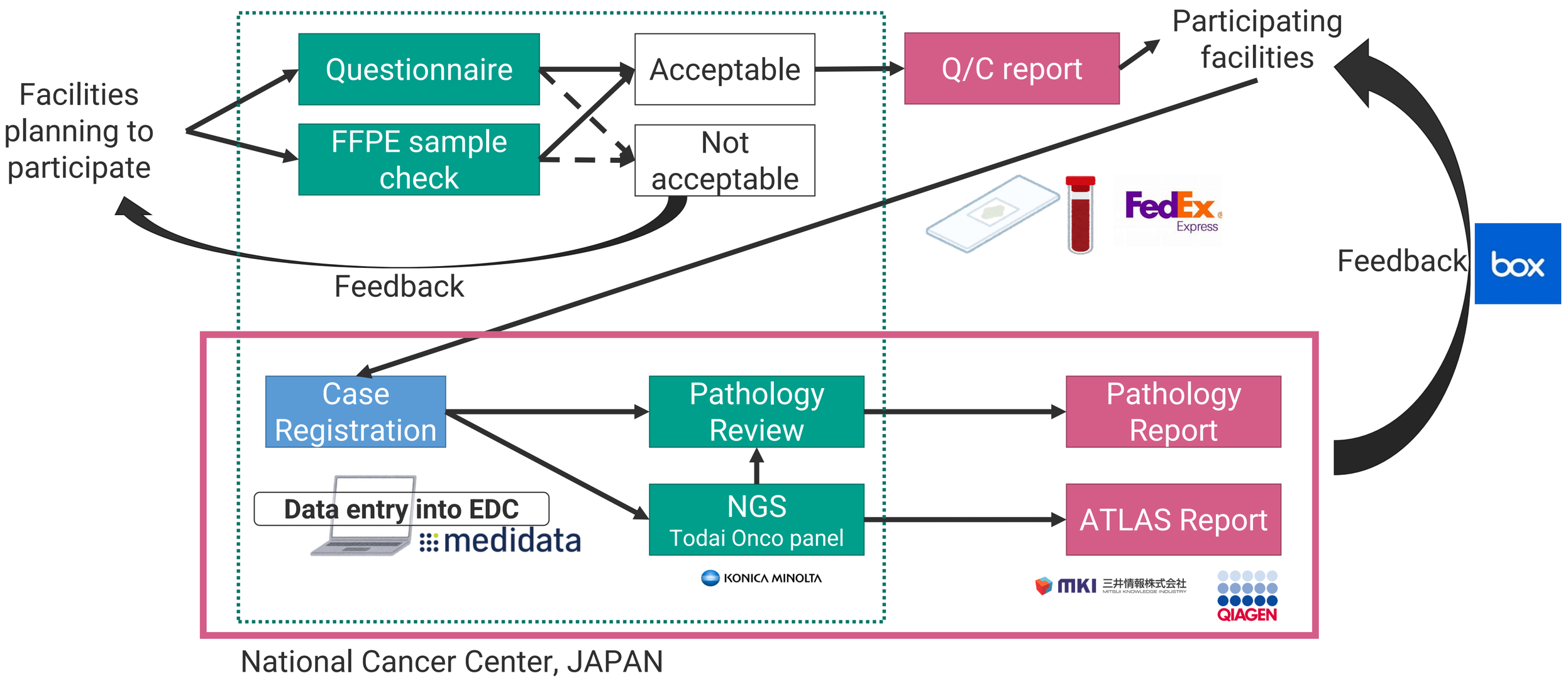
3.3 Assessment

Quality of FFPE specimen for molecular assays: Optimal

- Confirm results of each quality check of IHC, DNA, and RNA
- Check the questionnaire responses

→ Provide feedback on the results to the facility that submitted the specimens

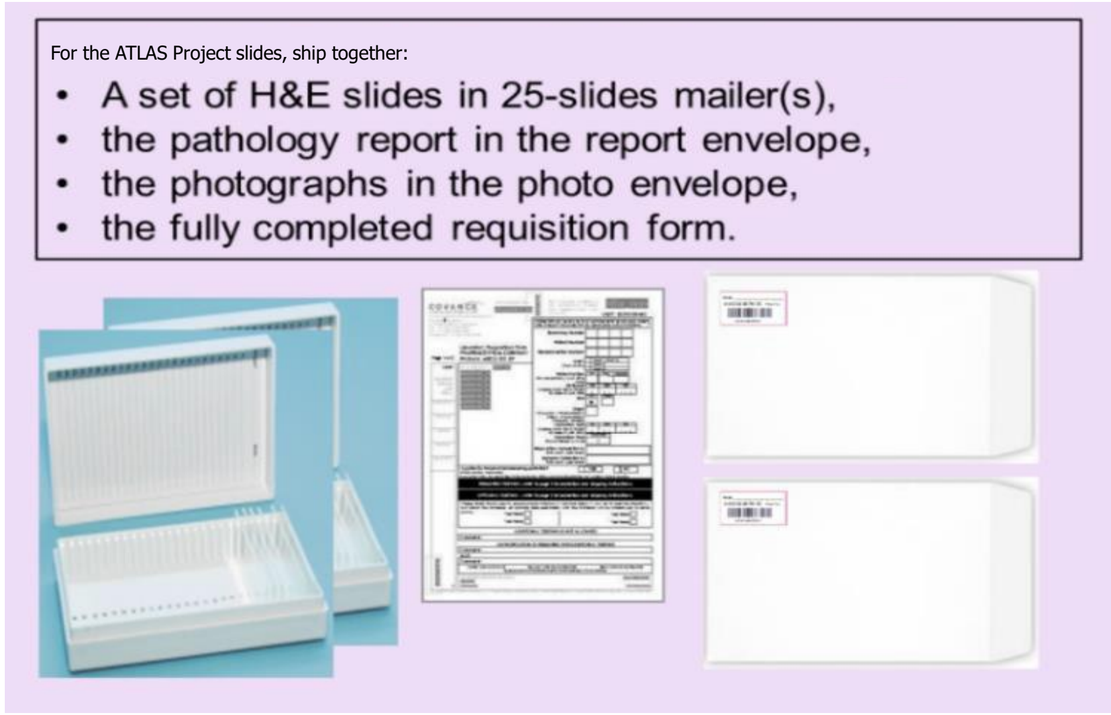
Overall Flow



Sample Shipment

- Prepared slides are to be placed in 25-slide mailers for ambient (i.e., room temperature) shipment to the following address with FedEx. The shipment must include the associated pathology report, the gross examination photographs if the sample is surgically resected, and the completed requisition form.

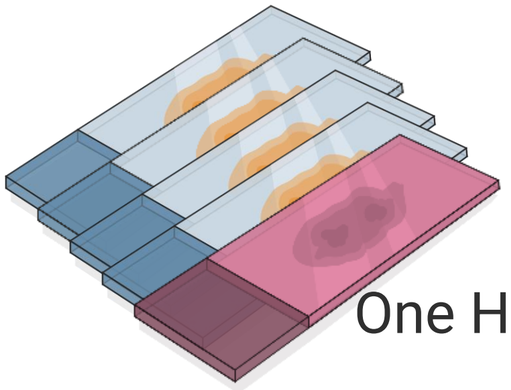
The address to be shipped:
Office, The ATLAS Project
Dept of Diagnostic Pathology,
National Cancer Center,
5-1-1 Tsukiji, Chuo-ku
Tokyo, 104-0045 Japan
Telephone: +81-3-3542-5201
FedEx Account ID: 795420312



How to prepare the tissue specimen

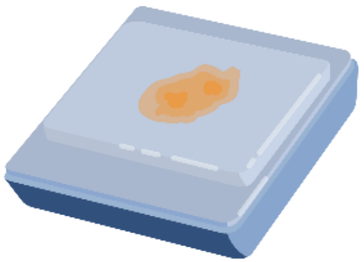
Anonymization

Before packing, FFPE block or unbaked glass slides should be anonymized using an oil-based pen.
FFPE samples must be coded by the same anonymization number as the Patient Registration No. that is given when registering the patient into the EDC: (2007-0001, 2007-0002…).
Label slide glass case with the provided sticker.



One HE stained slide

or



Included in the package

- Requisition Form
- Local Pathology Report (Copy)
- Clinical Abstract

*Sites will refer to the “Laboratory manual for MASTER KEY Asia” for details.
The questionnaire that each site pathology department fills out will also be reviewed by NCCH pathologists.

How to prepare the specimen Packaging

Packing kit

- IDS UN3373 Cardboard BOX
- Polyethylene Cushioning Pad
- Gel Wrap
- Non-woven Packet
- 95kPa Pressure Resistant Bio Pouch
- Bubble Wrap Bag

Prepare plastic case for tumor FFPE and blood collection tube

Same process as the Pathological quality check of ATLAS project

Please put them in 95kPa Pressure Resistant Bio Pouch

Put it in the Pouch and seal the lid.
The Star mark should be placed in the square mark

Use cushioning materials to secure the slide glass case and blood collection tube inside the box in right position.

Done!

Tasks Implemented During Pathology Review

- Observe representative sections of the tumor, referencing the clinical findings and the histopathological diagnosis from the participating facility
- Add tests such as immunostaining and ISH depending on the differential diagnosis
- Assign the name of the most probable histological diagnosis (if definitive diagnosis is not possible, the differential diagnosis group) and state opinions
- If analysis results of genetic abnormalities using NGS are obtained, also incorporate those results and summarize the final opinion and issue an “ATLAS pathology report”
- The NGS analysis results are issued separately as an “ATLAS Report”

Diagnostic Disagreement In Surgical Pathology

- When expertized pathologists reviewed 6171 cases at The Johns Hopkins Hospital, 86 cases (1.4%) were found to have discrepancies that would significantly change the treatment strategy and prognosis.

Kronz JD, et al. Cancer. 1999;86(11): 2426–2435.

- An investigation into 5629 cases at The University of Iowa Hospitals and Clinics found 132 cases (2.3%) of major disagreement and 507 cases (9.0%) of minor disagreement.

Manion et al. Am J Surg Pathol 2008;32: 732–737.

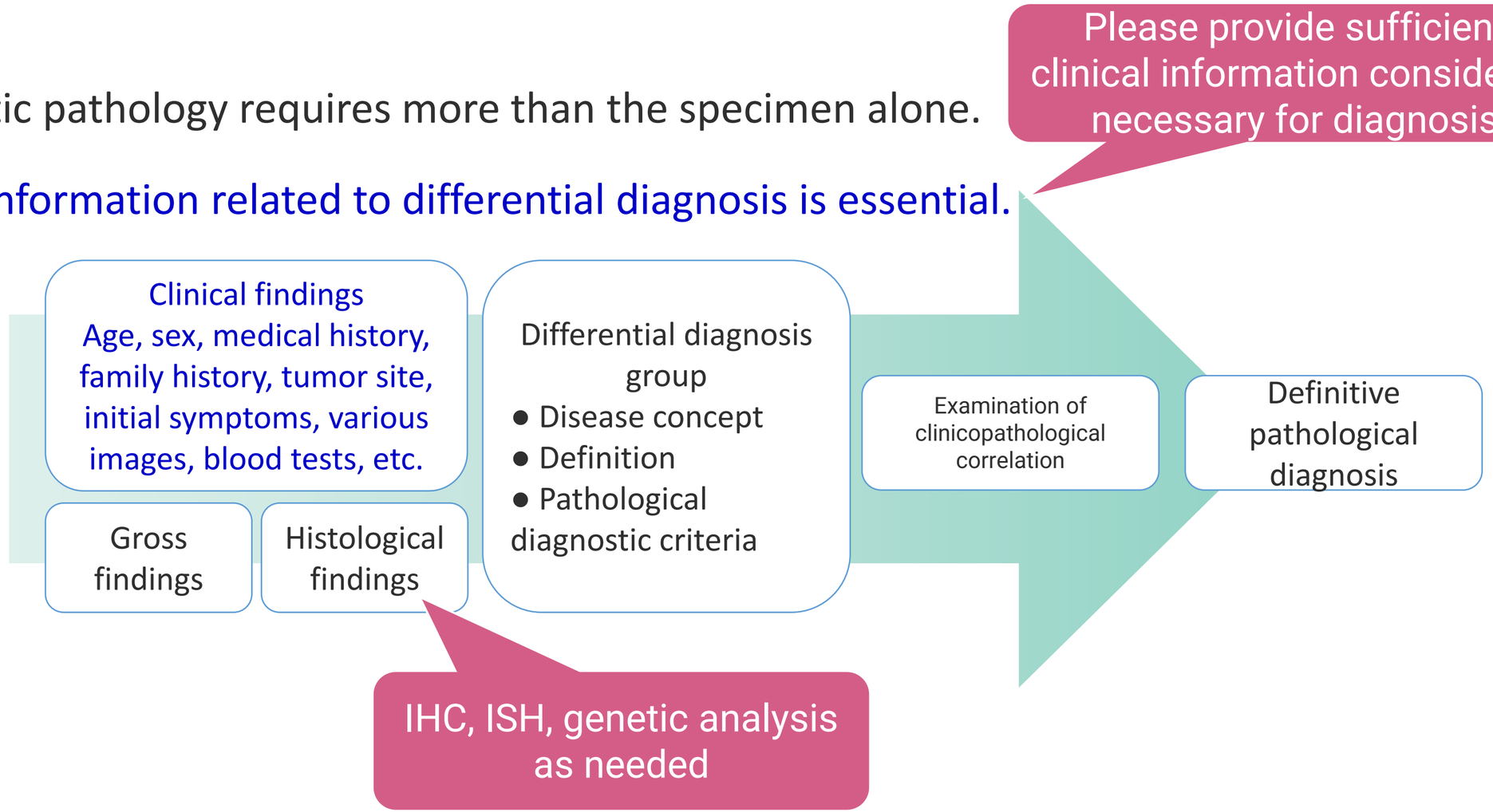
However, the discrepancy between diagnosticians is even greater with rare tumors

- A study that reviewed 603 cases of sarcoma found that only 207 cases (34.3%) had complete concordance in terms of diagnosis and grading.

Lehnhardt et al. J. Surg. Oncol. 2008;97: 40–43.

Sample Observation And Diagnostic Pathology

- Diagnostic pathology requires more than the specimen alone.
- Clinical information related to differential diagnosis is essential.



Flow Up To Pathology Review And Issuing NGS Report

Receive and check specimens

Representative section
Clinical summary
Pathology report

Diagnostic pathology
IHC and ISH are added as needed
NCCH pathologist is in charge

NGS
Targeted sequencing

NGS results are also considered for the pathology report

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ATLAS Pathology Report

Opinion on histological diagnosis

ATLAS report


ATLAS Report

ATLAS

About the ATLAS Report

1 Basic Information

NGS report on genetic abnormalities and therapeutic target

Turnaround time: approximately 1.5 months

Expected Outcomes From This Project

- Achieve diagnosis based on characteristics depending on morphological characteristics or tests such as immunohistochemical staining or obtain new evidence to support the diagnostic pathology performed in each facility, through review by experts specializing in the pathology of specific organs.
- Definitive diagnosis based on morphology is difficult, but diagnosis can be achieved through measures such as identification of fusion genes.

Recently, many disease units have been established on the basis of molecular biological abnormalities, and this trend is strong in rare cancers.

- Even if a definitive histopathological diagnosis is not achieved, molecular abnormalities that are therapeutic targets can be identified with NGS analysis.
- Establish a database linking the diagnostic pathology of rare cancers with genomic abnormalities in Asia.
- Searching for biomarkers is essential for future clinical trials, and ensuring the quality of FFPE samples forms the basis of these clinical trials.

→ Foundation of network for early drug development / international collaborative clinical trials

Take-home Messages

- The purpose of the MASTER KEY Asia project is to build a large-scale database linking accurate diagnostic pathology and molecular abnormalities that will form the basis of international collaborative investigator-initiated clinical trials for rare and common cancers in Asia.
- This project will verify the certification of each laboratory and check the sample preparation conditions and quality of pathology specimens, to obtain accurate diagnostic pathology results and suitable gene panel test analysis results.
- The results of pathology review and gene panel analysis for each case are returned to each facility as a report.
- Appropriate sample preparation and provision of information is necessary to implement pathology review and gene panel testing, so your cooperation is needed.