PhenoPath
A Quest Diagnostics Company

General specimen requirements

See test menu for test-specific requirements

For all cases, include the following (see test-specific specimen requirements below):

A. Completed PhenoPath requisition form

B. Pathology report corresponding to specimen (draft with at least the gross description is okay)

C. Relevant clinical history reports/information. Include all relevant clinical data—previous and presumptive diagnosis, pertinent medication/recent treatment (including dates of therapy), along with copies of any current or previous flow cytometry and/or cytogenetic reports

D. Current or previous flow cytometry and/or cytogenetic reports for hematopathology cases

E. Billing instructions and applicable information

F. Specimen for testing/consultation. ***Note:*** Primary specimen containers (the innermost container that actually holds the specimen, eg, blood tube, biopsy container, urine/sputum container) must be labeled appropriately with at least 2 patient identifiers and collection date. When multiple specimens from the same patient are collected for analysis, the source of the specimens must be clearly indicated on the primary container

G. H&E of specimen to be tested if possible (or applicable)

Specimen type	IHC/ histology	Flow cytometry [4,10,11]	FISH [5,10,11]	PCR or RT-PCR [5]	Direct IF: skin, mucosa, other	Indirect IF (serum) [6]	Chromosome analysis/ cytogenetics [10,11]	Storage/ transport
Formalin-fixed, paraffin-embedded tissue block/ cell block [1,2]	Tissue block with area of interest	N/A	Tissue block with area of interest	**See test menu for test-specific requirements**	N/A	N/A	N/A	Room temperature Use frozen ice pack
Formalin-fixed, paraffin-embedded unstained slides [3,7,8,13]	4 µm thickness, on adhesive slide	N/A	4 µm thickness, on adhesive slides		N/A	N/A	N/A	Room temperature Use frozen ice pack
Fresh tissue biopsy	In formalin	Finely minced tissue in RPMI [9]	In formalin		In Michel's (Zeus) transport media	N/A	In RPMI or tissue culture media	Room temperature Use frozen ice pack
Peripheral blood	N/A	**Preferred:** 3 mL in sodium heparin (green top) **Acceptable:** 3 mL in EDTA (purple top)	**Preferred:** 3 mL in sodium heparin (green top) **Acceptable:** 3 mL in EDTA (purple top)		N/A	At least 0.5 mL of serum; >1.0 mL is preferred	5 mL in sodium heparin (green top) [14]	Room temperature Use frozen ice pack
Bone marrow core/clot *,12	In formalin	In RPMI [9]	In formalin or in RPMI [9]		N/A	N/A	In tissue culture media or RPMI	Room temperature Use frozen ice pack
	In cell block; clot only	Preferred: 1-2 mL in sodium heparin (green top) Acceptable: 1-2 mL in EDTA (purple top)	Preferred: 1-2 mL in sodium heparin (green top) Acceptable: 1-2 mL in EDTA (purple top)		N/A	N/A	1-2 mL in sodium heparin (green top)	Room temperature Use frozen ice pack

Specimen type	IHC/ histology	Flow cytometry [4, 10, 11]	FISH [5, 10, 11]	PCR or RT-PCR[5]	Direct IF: skin, mucosa, other	Indirect IF (serum)[6]	Chromosome analysis/ cytogenetics [10, 11]	Storage/ transport
Malignant fluids	In formalin (prefer cell block)	In RPMI[9]	In formalin (prefer cell block) or in RPMI[9]	**See test menu for test-specific requirements**	N/A	N/A	N/A	Room temperature Use frozen ice pack
Fine needle aspirate (FNA)	In formalin (prefer cell block)	In RPMI[9]	In formalin (prefer cell block) or in RPMI[9]		N/A	N/A	N/A	Room temperature Use frozen ice pack
Cerebral spinal fluid (CSF)	In formalin (prefer cell block)	At least 1 mL of non-traumatically obtained CSF combined with an equal or greater volume of RPMI[9,a]	In formalin (prefer cell block) or in RPMI[9]		N/A	N/A	N/A	Room temperature Use frozen ice pack

a For bone marrow morphology, also provide fresh air-dried aspirate smears for evaluation.

1 Alternate fixative must be identified. Our testing is validated for formalin-fixed specimens. We have experience with and may have validated some tests to alternate fixatives; contact the laboratory if you have questions about a specific fixative.

2 Decalcified tissues: Reactivity may be compromised in ways impossible to predict. As with all histological procedures, thorough fixation prior to decalcification is required. Checking the endpoint of decalcification to avoid overtreatment also improves the likelihood of adequate immunoreactivity.

3 Formalin fixation >6 hrs and <72 hours is required for ER, PR; >6 hrs and <48 hrs for HER2 IHC and HER2 FISH studies.

4 For flow cytometry, please prepare a smear and send CBC results.

5 For FISH and PCR testing, refer to our website or contact the laboratory to determine the specimen requirements for the specific test(s) being requested.

6 Blood for IF studies should be drawn into a clot tube (red top), spun down and the serum separated from the clot.

7 Amyloid subtyping: Also submit two 8-10 µm sections (each on its own slide) for correlative Congo Red stain.

8 Contact the laboratory to find out how many slides to send, or if your slides are acceptable.

9 Do not use RPMI if it is cloudy, yellow, or beyond expiration date.

10 Green top tube = sodium heparin.

11 Purple/purple top tube = EDTA.

12 For bone marrow specimens, the non-decalcified clot is preferred for FISH and/or PCR studies.

13 Mole FISH: Also submit two 6µm sections (each on its own slide).

14 Patient should have WBC of 15,000 or higher, with 10% circulating immature myeloid or lymphoid blast cells.

** Provide bone marrow aspirate smears and/or stained slides in sealed containers with no exposure to formalin fumes.

*** The addition of RPMI precludes determination.

Paraffin-embedded specimen requirements

Our testing is validated for formalin-fixed specimens and identified by the type of fixative used on the requisition. Contact us if you have questions about a specific fixative.

Specimen collection

Collect specimen in accordance with your institution's policies and procedures. It is important that the specimen being submitted for testing contains the area of interest.

Specimen handling

Paraffin blocks: preferred (includes tissue blocks, as well as cell blocks from body fluids, FNAs, etc)

- Place block in glassine envelope—one block per envelope

Unstained slides:

- Insert into sturdy, top-loading slide-shipping container—tape container shut

Testing type	# of unstained slides required[a]	Notes
IHC	• 1 for each test requested • 3-4 additional unstained slides	For amyloid panel requests, submit one unstained 8-10 µm section or a Congo Red-stained slide.
FISH	5 (minimum)	Not all FISH tests can be performed on paraffin-embedded samples. Refer to our website or contact the laboratory to determine the specimen requirements for the specific test(s) being requested.
PCR	Contact us	The number of unstained slides needed depends on the amount of tumor in the block.

[a] Cut at 4 µm onto positively charged slides, 1 section per slide.

Specimen acceptability

Optimal:

- Specimen containing area of interest, fixed in formalin, embedded in paraffin, and appropriately labeled

Less than optimal:

- Melted or broken specimen
- Specimen not fixed in formalin
- Specimens that are not completely fixed

Unacceptable: Unacceptable specimens are accessioned, the specimen condition noted is documented in the case record, the sender is notified, the testing is canceled, documentation of test cancellation is generated, and the rejected specimen is returned.

- Specimen that does not belong to or cannot be confirmed as belonging to the patient
- Insufficient specimen for testing[b]
- Area of interest not present in specimen[b]

[b] May not be able to determine this until the laboratory work has been attempted.

Flow cytometry specimen requirements

RPMI information	
RPMI is the same as RPMI-1640/10% FBS (fetal bovine serum) and is supplied by PhenoPath	
Store:	Refrigerated at 2 °C–8 °C
Shelf life:	**2 Months** from the date prepared or until it turns cloudy or becomes yellow (refer to expiration date on vial)
Disposal:	In accordance with all applicable federal, state, and local regulations

Specimen collection

- **Peripheral blood:** In general, 5 to 10 mL of anticoagulated peripheral blood is adequate, although for patients with marked peripheral leukopenia of less than 1,000 white blood cells per µL, 10 to 20 mL should be considered*
- **Bone marrow aspirate:** 1 to 2 mL of anticoagulated bone marrow aspirate is adequate for high-quality, cellular specimens with little hemodilution by peripheral blood*
- **Bone marrow core biopsy:** In patients with inaspirable bone marrow and insufficient neoplastic cells in the peripheral blood to enable a definitive diagnosis, it may be possible to obtain sufficient cells for flow cytometry from a bone marrow core biopsy submitted unfixed in RPMI
- **Tissues:** Finely mince all tissues, except fine needle aspirations, to maximize exfoliation of cells into the medium, and then submerge in **RPMI-1640/10% FBS** in plastic vials (supplied by PhenoPath). **Under no circumstances should the cells be allowed to freeze**, since this will destroy cell membranes
- **Cerebrospinal fluid (CSF):** No less than 1 mL of non-traumatically obtained CSF should be reserved for flow cytometry. Due to the relative absence of nutrients in CSF, specimens to be sent for flow cytometry should be combined with an equal or greater volume of **RPMI** as soon as possible after the specimen is obtained (*Note:* The addition of **RPMI** precludes determination of the absolute leukocyte count in vivo in this aliquot of CSF)
- **Other body fluids, including bronchoalveolar lavage (BAL) fluids:** Combine such fluids with an equal or greater volume of **RPMI** prior to being sent

* For flow cytometry alone, sodium heparin anticoagulation (green top tube) is preferred, but EDTA anticoagulation (lavender top tube) is also acceptable. When flow cytometry and other studies are being requested, a separate heparin tube and EDTA-anticoagulated tube are preferred. When both flow cytometry and other studies are desired from a single specimen, EDTA anticoagulation is preferred.

Specimen handling

- Ensure primary specimen container is labeled with at least 2 patient identifiers
- Place primary specimen container in a biohazard bag and seal
- Ship specimens at ambient temperature

Specimen acceptability

Optimal:

- Collected <24 hrs prior to receipt

Less than optimal:

- Tissue placed in saline
- Specimen received in the lab >24 hours after it was obtained; specimen should be refrigerated and transported with a frozen ice pack

Unacceptable: Unacceptable specimens are accessioned, the specimen condition noted is documented in the case record, the sender is notified, the testing is canceled, documentation of test cancellation is generated, and the rejected specimen is returned.

- Specimen that does not belong to or cannot be confirmed as belonging to the patient
- Specimen placed in formalin

Skin and other specimens for direct IF

Specimen

Skin biopsy in Michel's (aka Zeuss) fixative, with or without patient serum

Michel's fixative information

Specimens in Michel's transport media can be refrigerated, or even held at room temperature, for as long as 2 weeks.

Store:	Ambient
Shelf life:	~1 year—see expiration date on vial
Disposal:	In accordance with all applicable federal, state and local regulations
Caution:	This substance is a skin and respiratory tract irritant

Specimen collection

In general, a punch or excisional biopsy from a fully developed lesion provides more information than an early lesion or a lesion in regression, with the following exceptions:

- **Vesicular, bullous, and pustular lesions**—For these, a very early lesion is necessary otherwise secondary changes may obscure essential features. The biopsy should be performed within a few millimeters of the edge of the blister and not farther than 1 cm away. A punch biopsy may be used, although the twisting motion may dislodge the epidermis, and cause false negative DIF results. A small excisional biopsy circumvents this problem. The specimen should include subcutaneous fat
- **Lupus erythematosus, lichen planus, vasculitis, and erythema multiforme**—In lesional lupus, lesions greater than 6 weeks old give better diagnostic yield, whereas in vasculitis, early lesions less than 24 hours old are optimal

Specimen handling

1. Completely submerge the specimen into Michel's fixative and tightly secure top
2. Label the primary specimen container with at least 2 patient identifiers (eg, complete patient name and your specimen number)
3. Place in biohazard bag and follow instructions for packing in general transport kit

Specimen acceptability

Optimal: Skin taken from appropriate site, and received completely submersed in transport media.

Less than optimal:

- Specimens received on saline-dampened gauze after an indeterminable amount of time
- Specimens that have been in transport media for more than 2 weeks
- Specimens received floating in large amounts of saline

Unacceptable: Unacceptable specimens are accessioned, the specimen condition noted is documented in the case record, the sender is notified, the testing is canceled, documentation of test cancellation is generated, and the rejected specimen is destroyed.

- Specimens that have been allowed to dry completely
- Received frozen in transport media or saline
- Specimen that does not belong to or cannot be confirmed as belonging to the patient

Serum for indirect IF

Specimen

Patient serum

Specimen Collection

Draw blood into a clot tube (red top).

Specimen handling

1. Spin down and separate serum from clot
2. Label primary specimen container (serum tube) with at least 2 patient identifiers (eg, complete patient name and your specimen number)
3. Place serum tube in biohazard bag and follow instructions for packing in general transport kit

Specimen acceptability

Optimal:

- Minimum volume of 3 mL of blood
- Specimens should be held at room temperature for <48 hours or refrigerated/ice packs for up to 7 days

Less than optimal:

- Specimen held at room temperature for longer than 48 hours
- Less than 3 mL of serum

Unacceptable: Unacceptable specimens are accessioned, the specimen condition noted is documented in the case record, the sender is notified, the testing is canceled, documentation of test cancellation is generated, and the rejected specimen is destroyed.

- Specimen received frozen
- Specimen that does not belong to or cannot be confirmed as belonging to the patient

Salt split skin

Specimen

Skin biopsy in Michel's (aka Zeuss) fixative, with or without patient serum

Michel's fixative information	
Specimens in Michel's transport media can be refrigerated, or even held at room temperature, for as long as 2 weeks.	
Store:	Ambient
Shelf life:	~1 year—see expiration date on vial
Disposal:	In accordance with all applicable federal, state and local regulations
Caution:	This substance is a skin and respiratory tract irritant

Specimen collection

Skin punch biopsies or bisected punch biopsies are preferred.

Specimen handling

1. Completely submerge the specimen into Michel's fixative (an ammonium sulfate-based "holding media") and tightly secure top. Specimens in transport media can be refrigerated, or even held at room temperature, for as long as 2 weeks
2. Label primary specimen container with at least 2 patient identifiers (eg, complete patient name and your specimen number)
3. Place serum tube in biohazard bag and follow instructions for packing in general transport kit

Specimen acceptability

Optimal: Skin taken from appropriate site, and received completely submersed in transport media.

Less than optimal:

- Specimens received on saline-dampened gauze after an indeterminable amount of time
- Specimens that have been in transport media for more than 2 weeks
- Specimens received floating in large amounts of saline
- A negative or variable antibody reaction pattern found, with appropriate quality control

Unacceptable: Unacceptable specimens are accessioned, the specimen condition noted is documented in the case record, the sender is notified, the testing is canceled, documentation of test cancellation is generated, and the rejected specimen is destroyed.

- Specimens that have been allowed to dry completely
- Received frozen in transport media or saline
- Specimen that does not belong to or cannot be confirmed as belonging to the patient

Chromosome analysis: neoplastic specimen requirements

Specimen requirements and shipping

All specimens must be labeled with patient's name and be accompanied by a completed requisition form. All samples should be kept at room temperature and transported to PhenoPath with a frozen ice pack with minimum delay. Please call 1.888.927.4366 if you have any questions.

Specimen type	Chromosome analysis	FISH when run in conjunction with chromosome analysis	Storage/transport
Bone marrow aspirate	In green top, 1-2 mL	In green top, 1-2 mL Acceptable: lavender top	Room temperature; transport with frozen ice pack
Solid tumor tissue/ lymph node	In RPMI or tissue culture media	In RPMI or tissue culture media	Room temperature; transport with frozen ice pack
Peripheral blood-neoplastic	In green top, 5 mL, patient should have WBC of 15,000 or higher, with 10% circulating immature myeloid or lymphoid blast cells	In green top, 5 mL Acceptable: lavender top	Room temperature; transport with frozen ice pack

Solid tissue

All solid tissue samples should be collected aseptically and transported in tissue culture media or Hank's balanced salt solution. Do NOT put in water, fixative, formalin, or saline. Please keep sample at room temperature.

- Skin Biopsy/Solid Tissue: 1-3 mm^3 or more tissue. Label tube with tissue type or origin

Neoplasia

- **Bone marrow:** Aspirate 1-2 mL bone marrow into a sterile syringe containing 0.1 mL preservative free sodium heparin; invert syringe to mix and transfer to a 3 mL preservative-free sodium-heparin (green-top) vacutainer tube
- **Leukemic peripheral blood:** Patient should have WBC of 15,000 or higher with approximately 10% circulating immature myeloid or lymphoid blast cells. Collect 5 mL of peripheral blood in a preservative-free sodium-heparin (green top) vacutainer tube
- **Solid tumor tissue:** >5 mm^3 representative tumor tissue collected under aseptic conditions and transported in sterile tissue culture media
- **Lymph node biopsy:** >5 mm^3 tumor biopsy collected under aseptic conditions and transported in sterile tissue culture media

Fluorescence in situ hybridization (FISH) when ordered in conjunction with chromosome analysis

- FISH studies are indicated when classic cytogenetics alone cannot resolve an abnormality. Specimen collection is as described previously for the tissue to be studied

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