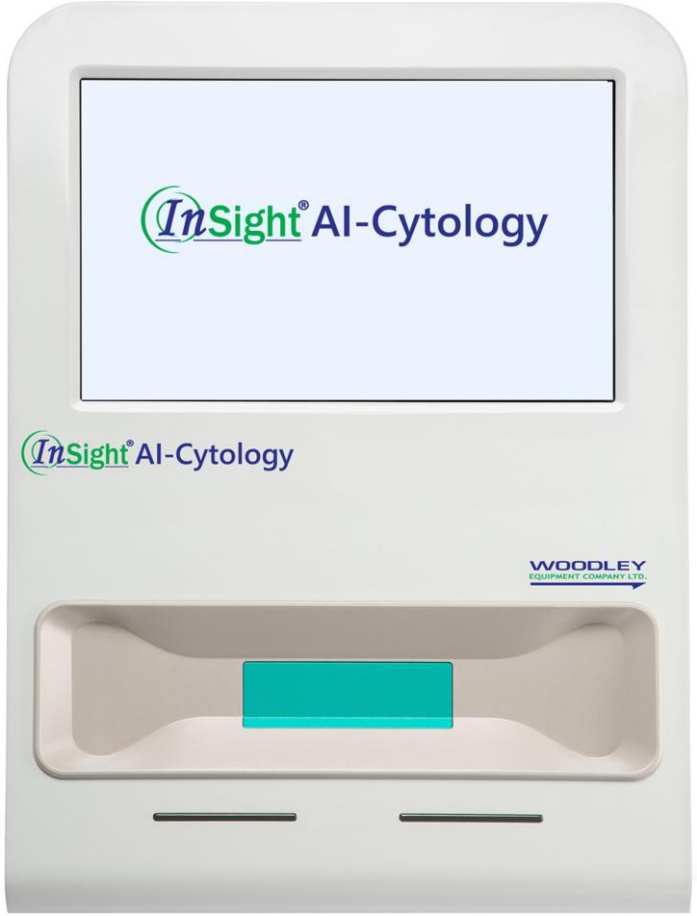


Interpretation of Fluid Parameters

WOODLEY
EQUIPMENT COMPANY LTD.

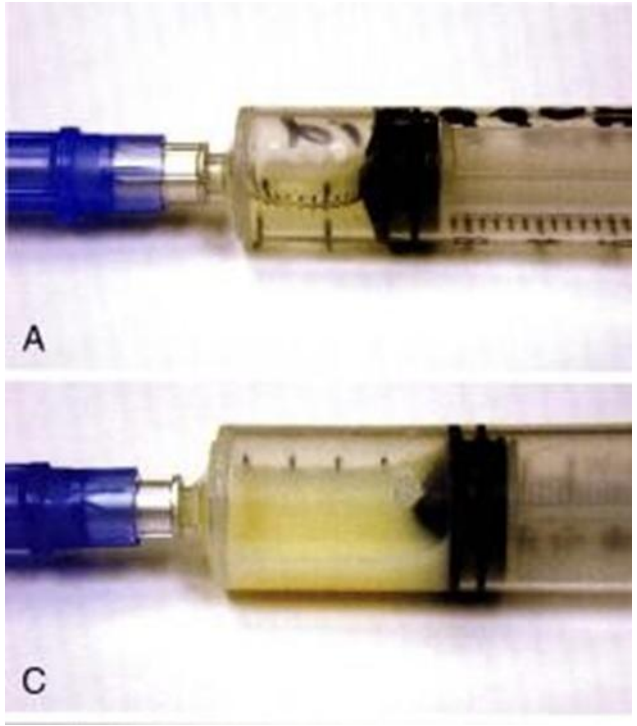
InSight® AI-Cytology

Fluid Testing

Purpose of Fluid Testing

It provides useful information for the diagnosis, prognosis and treatment of diseases causing pleural and abdominal effusion.

It also helps doctors classify the types of effusion.



InSight AI-Cytology Fluid Parameters

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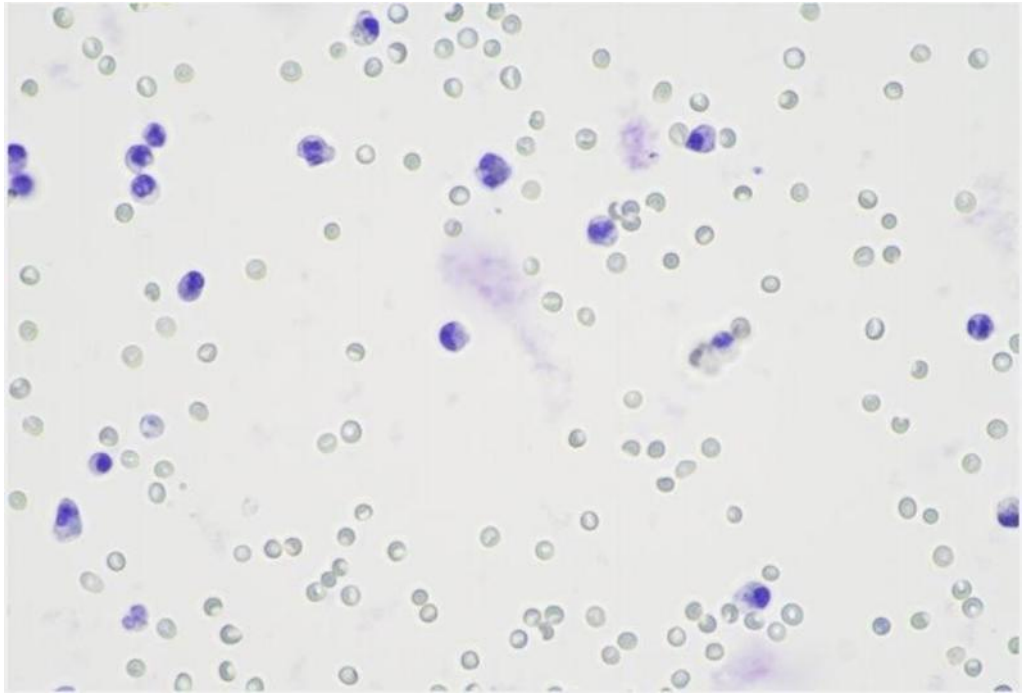
TNCC
Inflammatory cells, granulocytes, lymphocytes, macrophages, degenerative neutrophils, mesothelial cells, and unclassified nucleated cells can be detected.

RBC
RBC count and PCV can be detected.

Microorganism
Cocci and bacilli can be detected.



Fluid Report

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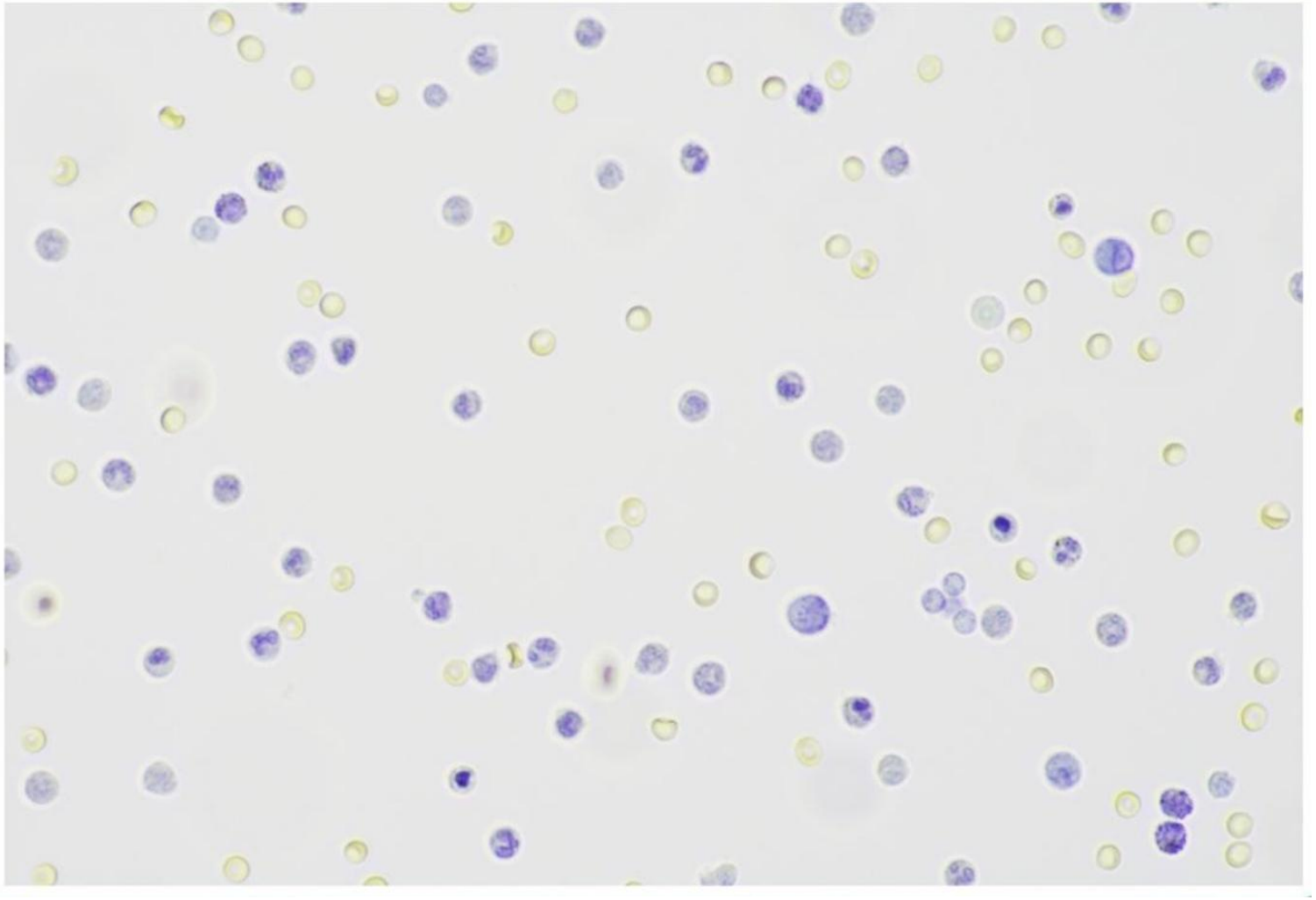
Parameters

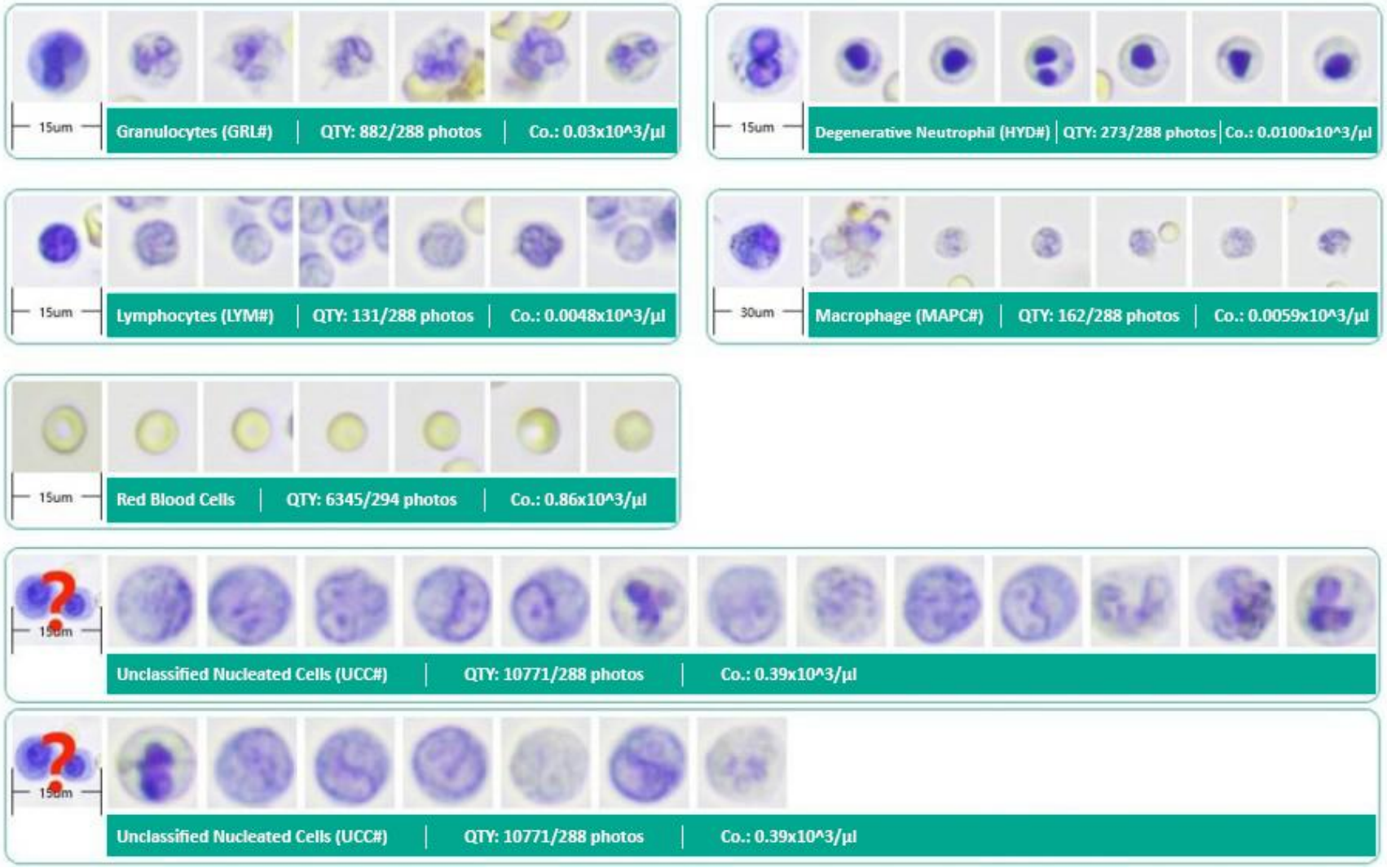
Detection Items	Result	Unit	Reference	Negative	Positive
1. Nucleated Cell					
1-1. Total Nucleated Cell Count (TNCC#)	0.44	10^3/μl	0-0		+
1-2. Inflammatory Cell Count (INC#)	0.04	10^3/μl	0-0		+
1-3. Total Granulocyte Count (GRL#)	0.03	10^3/μl	0-0		+
1-4. Neutrophils (NEU#)	0.03	10^3/μl	0-0		+
1-5. Degenerative Neutrophil Count (HYD#)	0.01	10^3/μl	0-0		+
1-6. Neutrophils (NEU%)	100.00	%			
1-7. Degenerative Neutrophil Count (HYD%)	30.99	%			
1-8. Lymphocytes (LYM#)	0.0048	10^3/μl	0-0		+
1-9. Macrophage (MAPC#)	0.0059	10^3/μl	0-0		+
1-10. Granulocyte Percentage (GRL#/TNCC#)	7.39	%			
1-11.Lymphocytes Percentage (LYM#/TNCC#)	1.10	%			
1-12. Macrophage Percentage (MAPC#/TNCC#)	1.36	%			
1-13. Mesothelial Cell Count (MEC#)	0.00	10^3/μl	0-0	−	
1-14. Phagocytic Cell (PHC#)	0.00	10^3/μl	0-0	−	
1-15. Unclassified Nucleated Cells (UCC#)	0.39	10^3/μl			
2. Erythrocytes					
2-1. Red Blood Cells (RBC#)	0.86	10^3/μl	0-0		+
2-2. Pack Cell Volume (PCV%)	0.0054	%			
3. Microorganisms					
3-1. Rods (BAC#)	0.00	/μl	0-0	−	
3-2. Cocci (COS#)	0.00	/μl	0-0	−	

Fluid Report



Cellular Distribution Graph

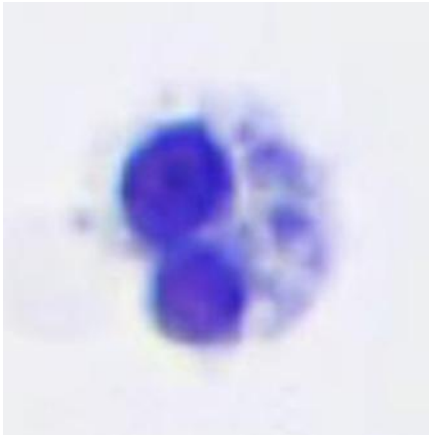
Fluid Report



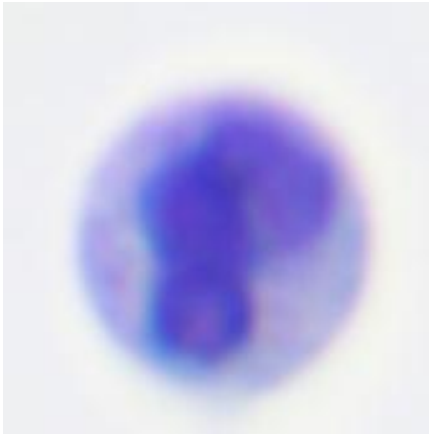
TNCC Interpretation

Granulocytes

- ❑ They are mostly found in inflammatory effusions.
- ❑ They are divided into degenerative and non-degenerative neutrophils.
- ❑ The presence of a large number of degenerative neutrophils suggests septic exudation.



Degenerative Granulocytes
(nuclear cytoplasmic swelling)↑

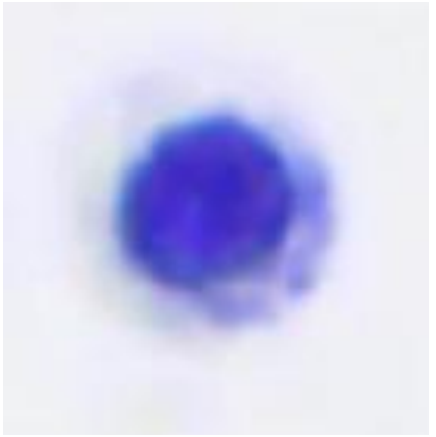


Non-degenerative Granulocytes
(tight nuclear and cytoplasmic)↑

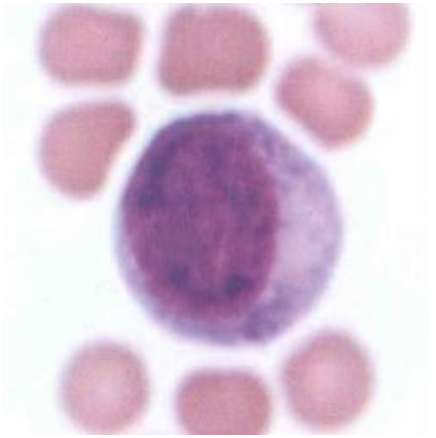
TNCC Interpretation

Lymphocytes

- They are often found in chylous exudates.
- They can also be secondary to the exudate of lymphoma.
- Reactive lymphocytes may be present in the inflammatory exudate.



Lymphocyte↑

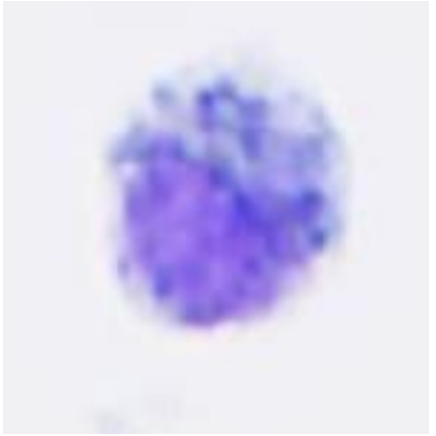


Reactive Lymphocytes↑

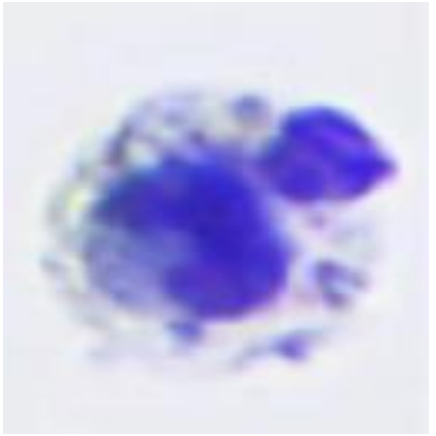
TNCC Interpretation

Macrophages

- Monocytes become macrophages when they are released from blood vessels into tissues.
- The nuclear and cytoplasmic staining is often lace-like and the cytoplasm is often vacuolar.
- They often contain phagocytic material such as bacterial or cellular debris.



Macrophage↑

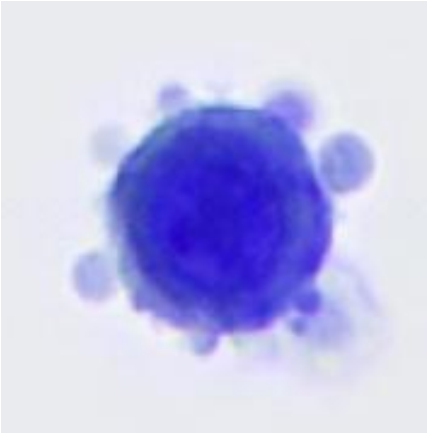


Phagocyte-like Macrophages↑

TNCC Interpretation

Mesothelial Cells

- Mesothelial cells are derived from the pleural, peritoneal and pericardial cavities, and the visceral layers of these body cavities.
- Mesothelial cells are prone to activation, reaction or proliferation in response to inflammation or accumulation of fluid.
- Reactive mesothelial cells have a variety of similar manifestations with malignant tumour cells; attention should be paid to their identification.



Mesothelial Cell↑

RBC & Microorganisms

- The presence of red blood cells in the exudate is associated with bleeding or contamination with iatrogenic blood.
- The presence of bacteria in the exudate suggests infectious exudate.

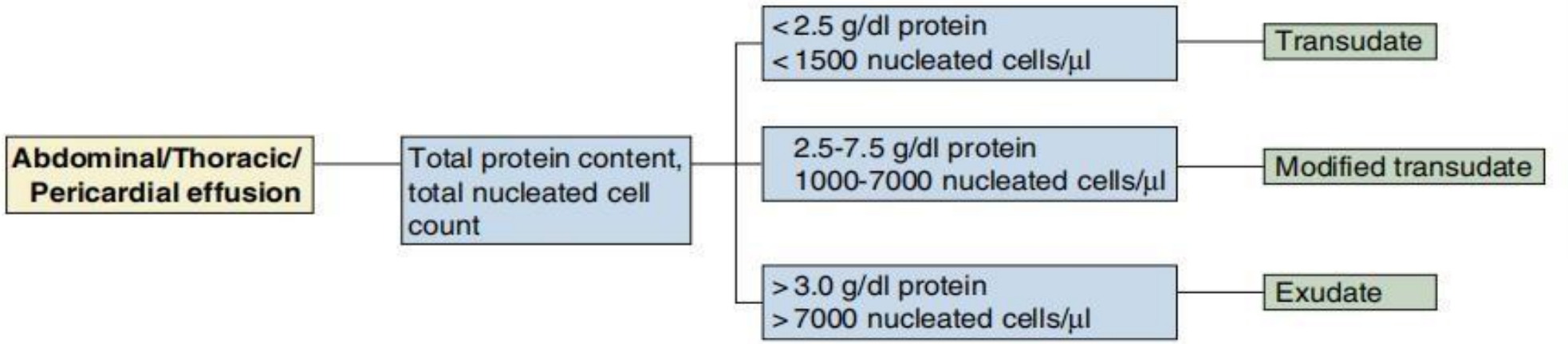
RBC

Bacilli

Cocci

Effusion Classification – Transudate

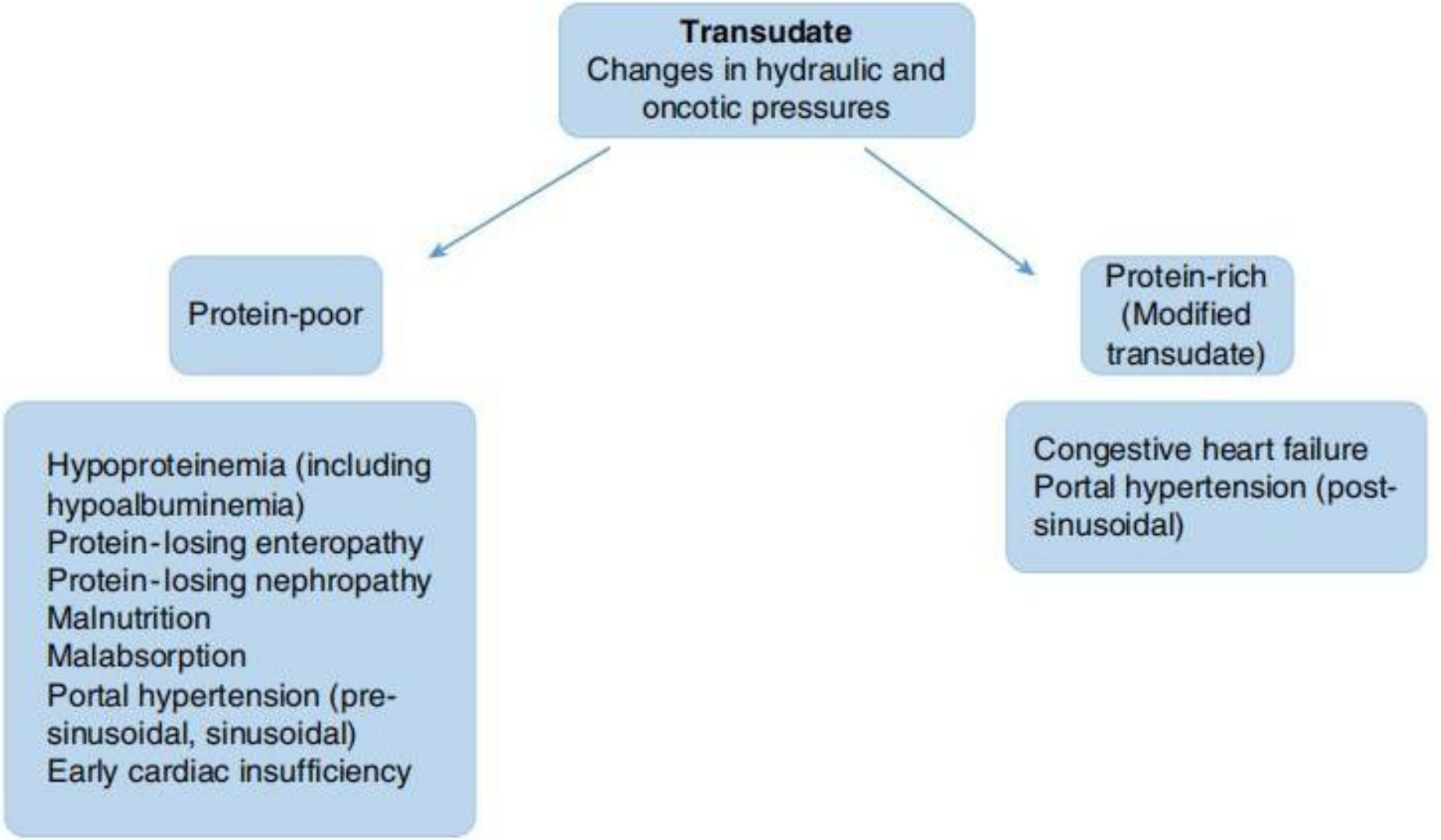
- The protein concentration (<2.5 g/dL) and cell count (<1500 µl) of the effusion are usually clear and colourless.
- The cells in the effusion are mainly monocytes, mesothelial cells and a small amount of non-degenerate neutrophils.



- The exudates were classified as exudate, modified transudate, or transudate based on total protein (TP) and nucleated cell count (TNCC)

*Refer from Diagnostic Cytology and Haematology of the Dog and Cat, Rick L. Cowell and Amy C. Valenciano P235

Transudate & Modified Transudate



*Refer from Diagnostic Cytology and Haematology of the Dog and Cat, Rick L. Cowell and Amy C. Valenciano P236

Effusion Classification – Transudate

Simple Transudate

- The cell count in a simple transudate is low (i.e., <1000 dL), the protein (TS) concentration is <2.5 g/dL and the specific gravity (SG) is <1.017.
- Typical symptoms include ascites caused by portal hypertension and low albumin levels, as well as liver dysfunction.
- It is accompanied by severe hypoproteinaemia associated with sodium and water retention in protein-losing nephropathy (PLN) and protein-losing enteropathy (PLE).
- Medical fluid overload or peritonitis caused by bladder or ureter rupture.

Effusion Classification – Transudate

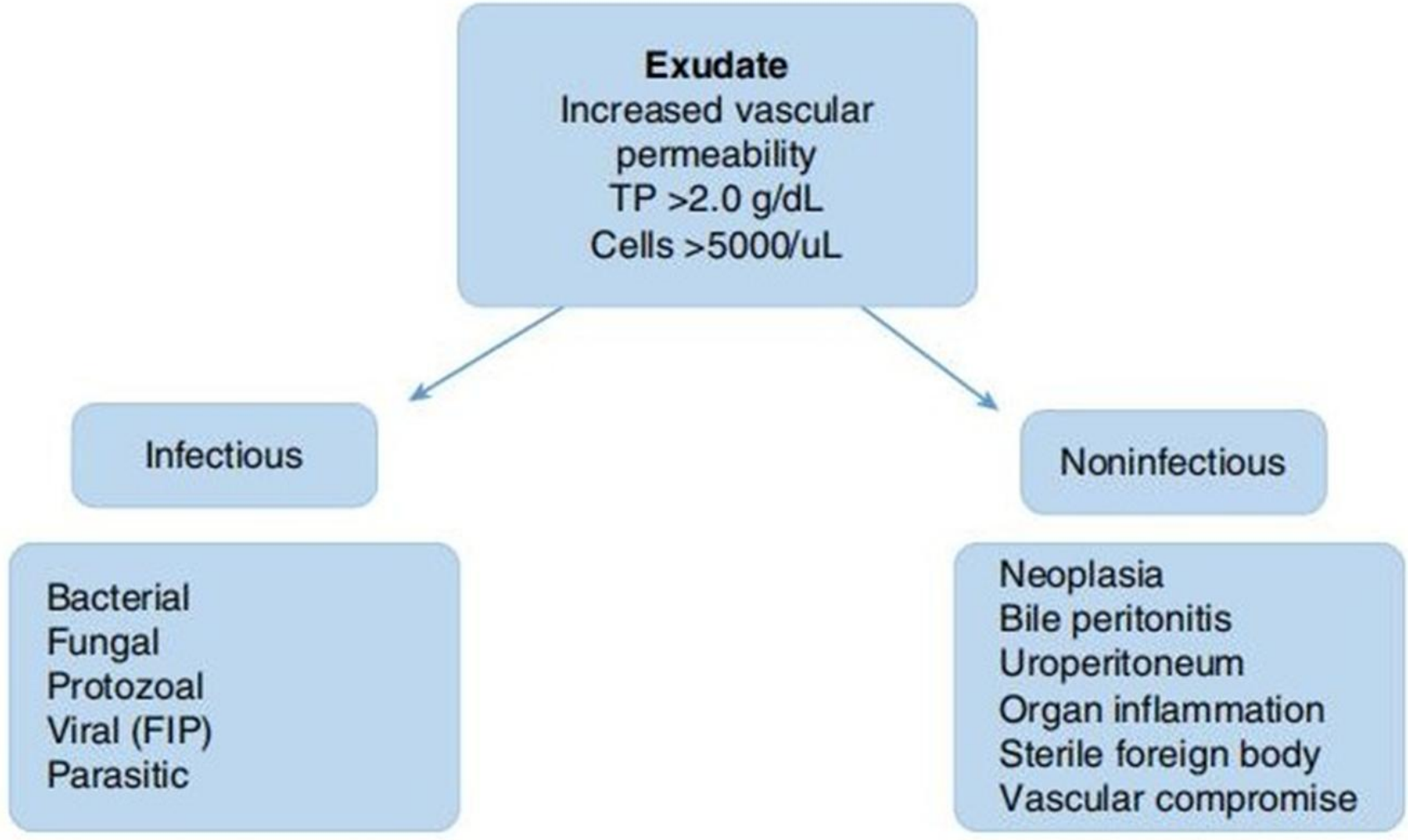
Modified Transudate

- The protein (TS) concentration in modified exudates is higher than that of simple exudates (usually 2.5 g/dL – 7.5 g/dL) and the specific gravity is usually greater than 1.017. The cell count is typically between 1000µl – 7000µl and the cells are often enriched with mesothelial cells.

- Modified exudates reflect leakage from normal or non-inflammatory blood vessels due to increased hydrostatic pressure in capillaries or obstruction of lymphatic vessels.

- Modified exudate may be related to tumours and many other conditions that cause exudative effusions, as well as peritonitis caused by bladder or ureter rupture.

Effusion Classification – Exudate



*Refer from Diagnostic Cytology and Haematology of the Dog and Cat, Rick L. Cowell and Amy C. Valenciano P236

Effusion Classification – Exudate

- Fluid with high protein concentration (TS >3.0 g/dL) and high specific gravity (>1.025). Increased cells (>7000µl), mainly neutrophils and macrophages.
- Cytologic examination of the effusion immediately before bacterial and fungal culture is important to identify septic exudates.

Septic Exudate

- The septic exudate may have been caused by systemic sepsis.
- Such as pleuropneumonia, gastrointestinal diseases, intestinal perforation etc.
- There may also be iatrogenic infection due to body cavity puncture.

Effusion Classification – Exudate

Chylous Exudate

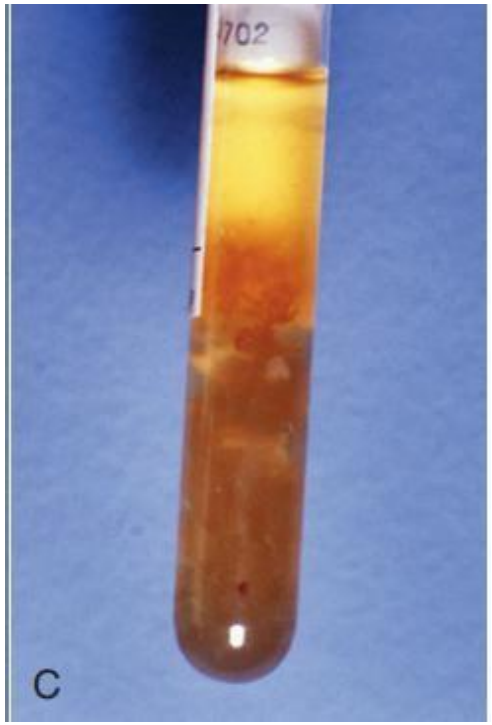
- The total protein TS concentration of empyema is greater than 2.5 g/dL and the specific gravity is greater than 1.018. It is characterised by the predominance of mononuclear cells (lymphocytes) or large numbers of neutrophils.

- Effusions of chyle reflect rupture of the thoracic duct or small lymphatic vessels, obstruction due to infiltration by a tumour in the lymphatic vessels or drainage lymph nodes (such as lymphoma, thymoma), inflammation or tumours in the mediastinum, obstruction of lymphatic vessels due to hernias or pericardial tamponade, lung lobe torsion, congenital disorders (such as lymphatic hypertrophy) and cardiac disease.

Feline chylothorax effusion, milky white

FIP

- FIP can cause abdominal and pleural effusions, and occasionally pericardial effusions in cats.
- The exudate is odourless, straw-coloured to golden, viscous and may contain cellulose filaments.
- The exudate type of FIP may be exudate or modified transudate.
- Most exudates have very high protein concentrations (>4 g/dL).
- The main cell composition was non-degenerative neutrophils and macrophages.
- The cytology of pleural effusion and ascites can assist in the diagnosis of FIP but cannot be used as a direct diagnostic basis.



Pleural effusions of FIP in cats
Nonpurulent exudate

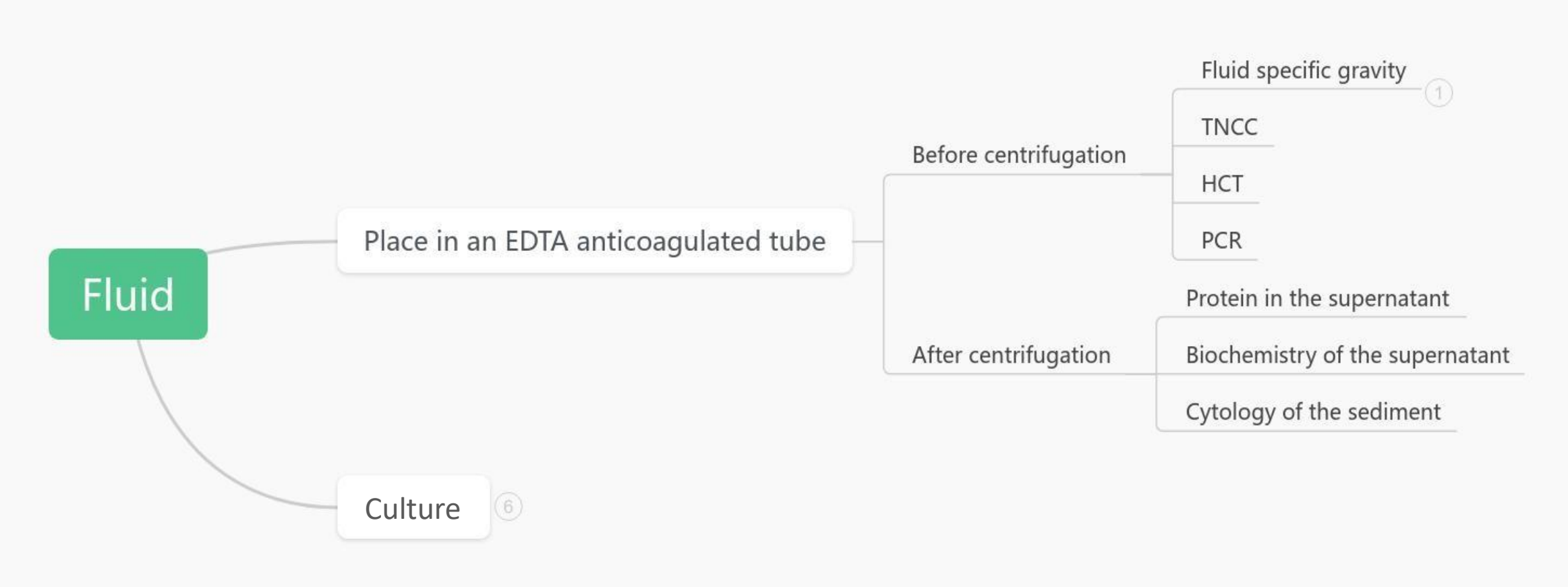
Chylothorax

- Chylous exudates are rich in chylomicrons, which are rich in triglyceride lipoproteins.
- Chylous exudates in dogs and cats often occur in the form of bilateral pleural exudates with less ascites.
- The chylous exudate is mainly composed of a large number of mature small lymphocytes with a small number of plasma cells and macrophages. Odourless, milky white, turbid yellow or turbid pink in colour.
- There may be an increase in neutrophils in the chyle of the pleural cavity.
- Bacterial infection of chylous exudate is rare because of the bacteriostatic effect of fatty acids in chyle.

References

- Diagnostic Cytology and Haematology of the Dog and Cat, Rick L. Cowell & Amy C. Valenciano
- Small Animal Clinical Diagnosis by Laboratory Methods, Michael D. Willard & Harold Tvedten
- Small Animal Internal Medicine, Richard W. Nelson & C. Guillermo Couto
- Clinical Atlas of Small Animal Cytology and Haematology, Andrew G. Burton

Conventional Fluid Testing Process



Fluid Collection

- Preparation – A 5ml syringe is often used.
- Usually 3ml – 5ml of liquid stored in a sterile EDTA (purple headed tube) anticoagulant tube is required for analysis.
- Liquid Analysis – It should be analysed immediately to avoid neutrophil degeneration caused by long-term storage of samples in EDTA to qualitative and guide further diagnosis (such as bacterial culture).

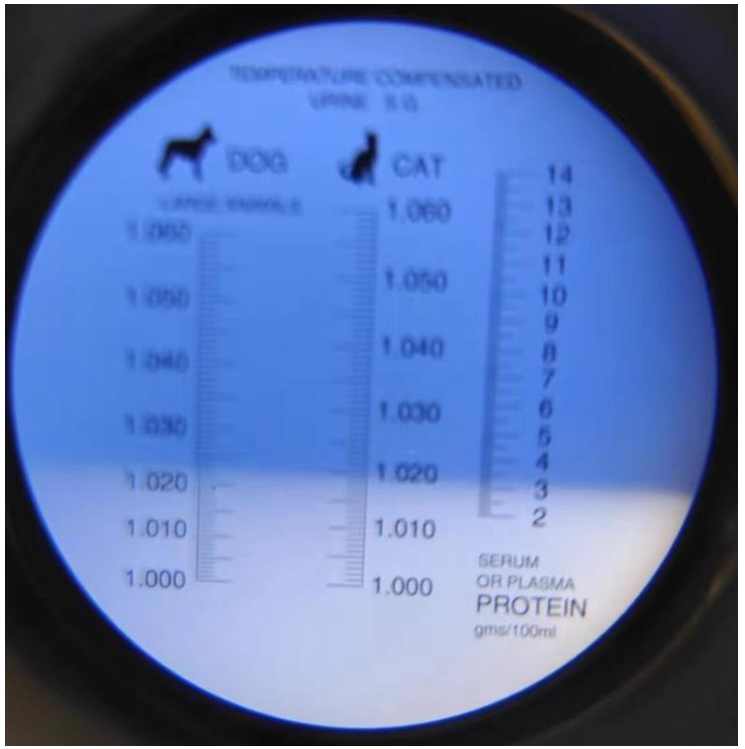
Fluid Testing Process

Total Protein Measurement

The total protein concentration of the fluid combined with the nucleated cell count allows the classification of the exudate as transudate, modified transudate or exudate. It also allows for the assessment of the severity of inflammation.

The total protein amount can be measured biochemically or estimated by refractometry. A refractometer is recommended to measure total protein, which is simple and accurate.

*If the liquid is cloudy due to the suspension of non-protein particles (lipoprotein, urea, cholesterol and glucose) caused by refractometer error, it is best to take the centrifuged supernatant to determine. The chylous or lipemic fluid may not be completely separated for refractometric or chemical measurement of total protein.

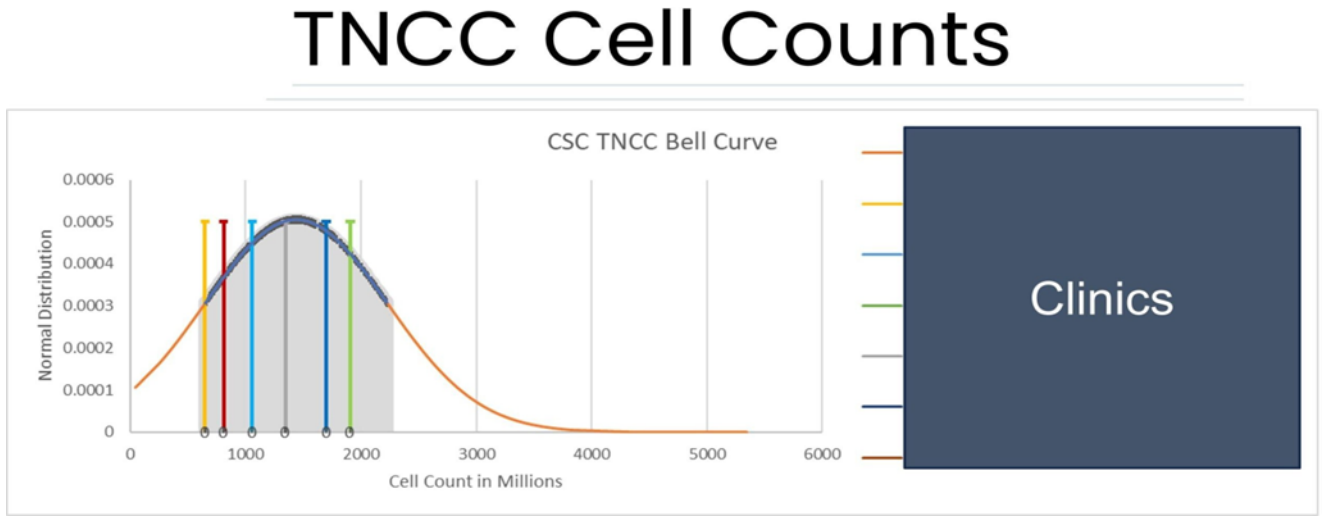


Fluid Testing Process

TNCC Measurement of Nucleated Cell Count

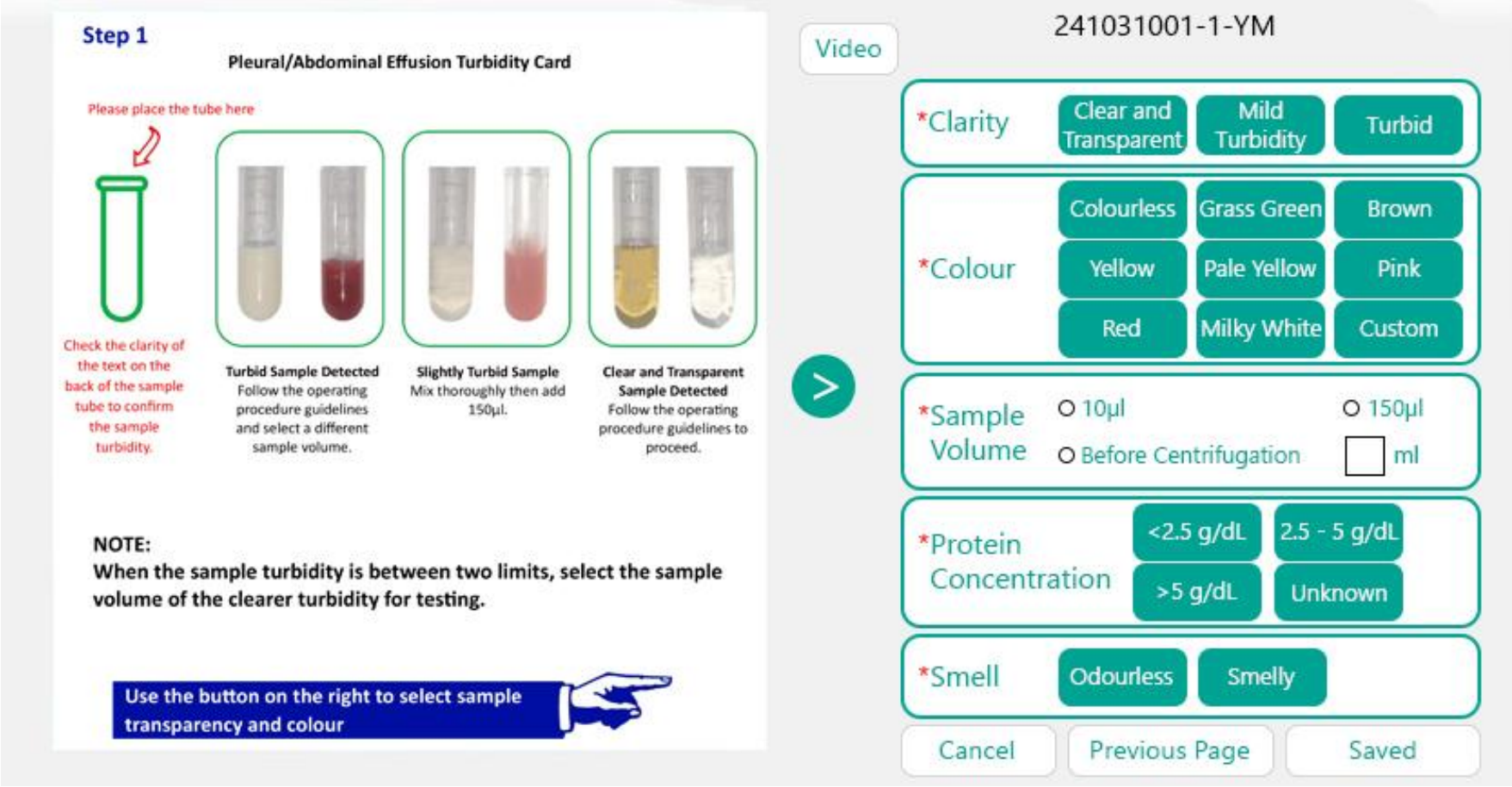
Accurate nucleated cell count can be obtained by using a blood cell analyser. However, cell volume, cell debris and non-cellular debris may cause errors in the calculation of the blood cell analyser.

Using the InSight AI-Cytology fluid function to count and classify nucleated cells, instead of traditional CBC+ artificial cytology, the total number of nucleated cells, the classification of inflammatory cells and the HCT of effusion were directly obtained.



Fluid Operation

A refractometer was used to determine the concentration of protein in fluid. According to the concentration, select the corresponding protein concentration range.



Fluid Testing Process

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Pleural/Abdominal Effusion Turbidity Card

Please place the tube here

Check the clarity of the text on the back of the sample tube to confirm the sample turbidity.

Turbid Sample Detected
Follow the operating procedure guidelines and select a different sample volume.

Slightly Turbid Sample
Mix thoroughly then add 150µl.

Clear and Transparent Sample Detected
Follow the operating procedure guidelines to proceed.

Fluid Testing Process

Turbid Sample: 10µl

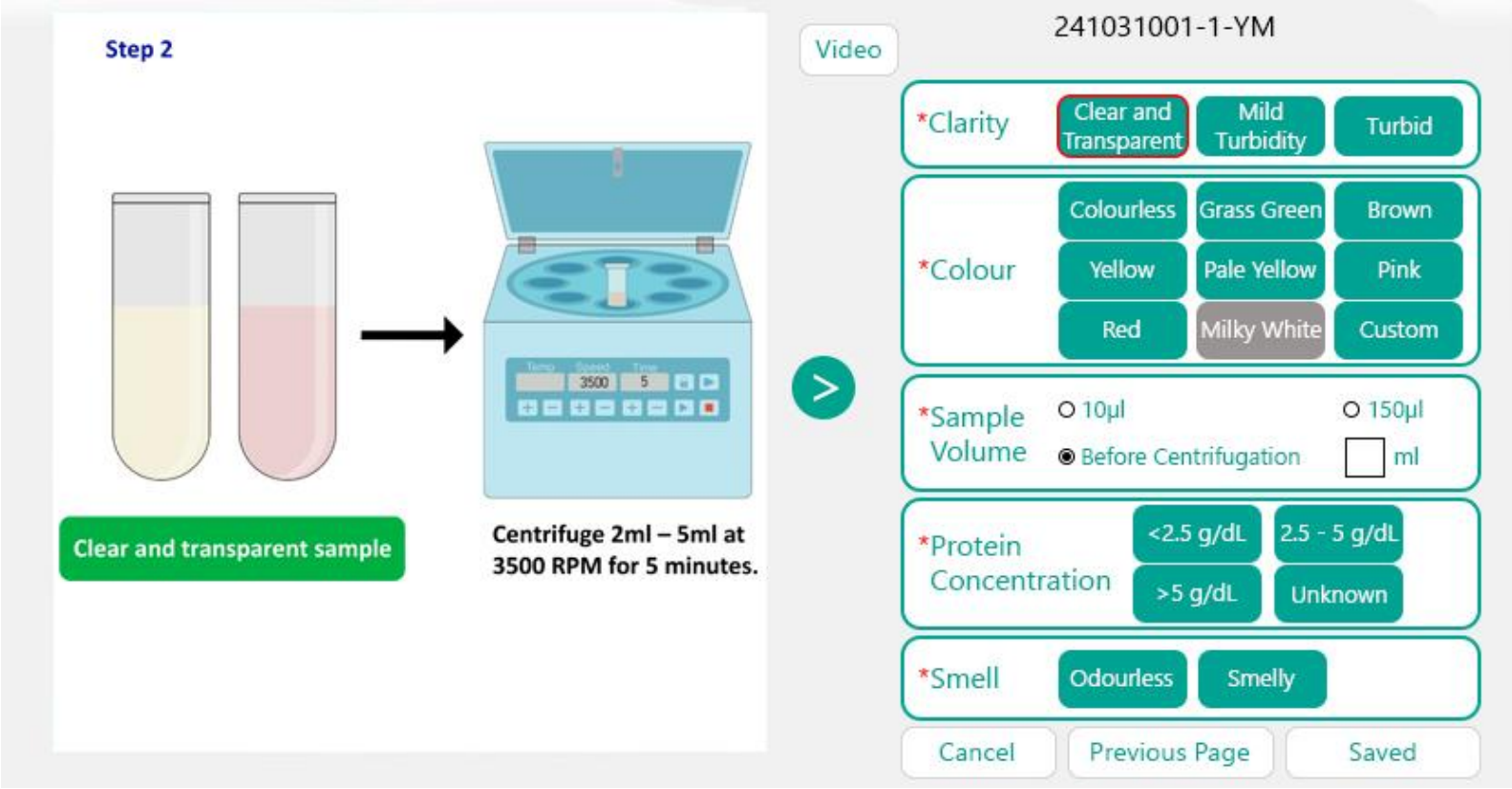
Mild Turbidity: 150µl

Clear Sample: Remove 2ml – 5 ml into an empty tube. Centrifuge at 3500 RPM for 5 minutes and take 150µl.

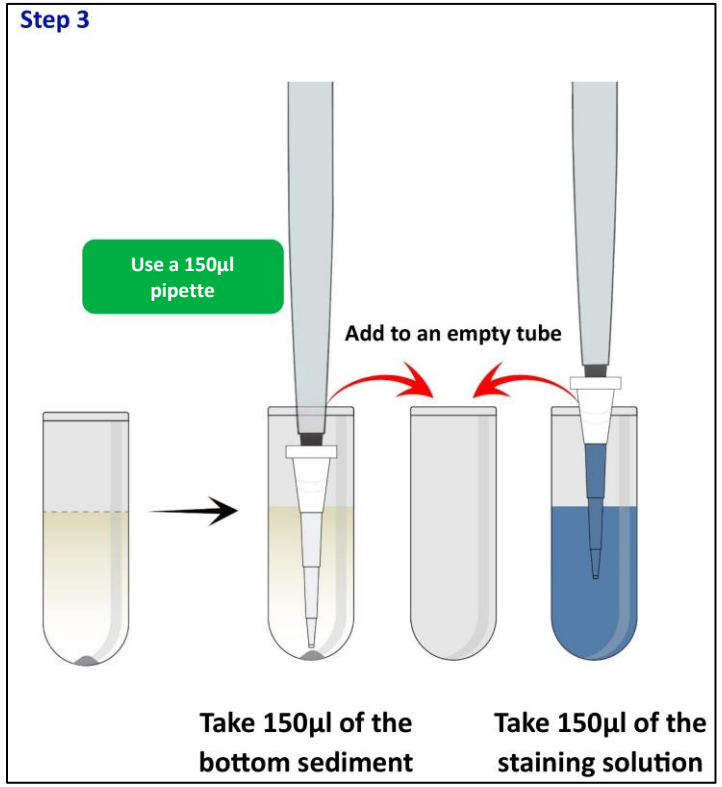
Clear and Transparent Sample

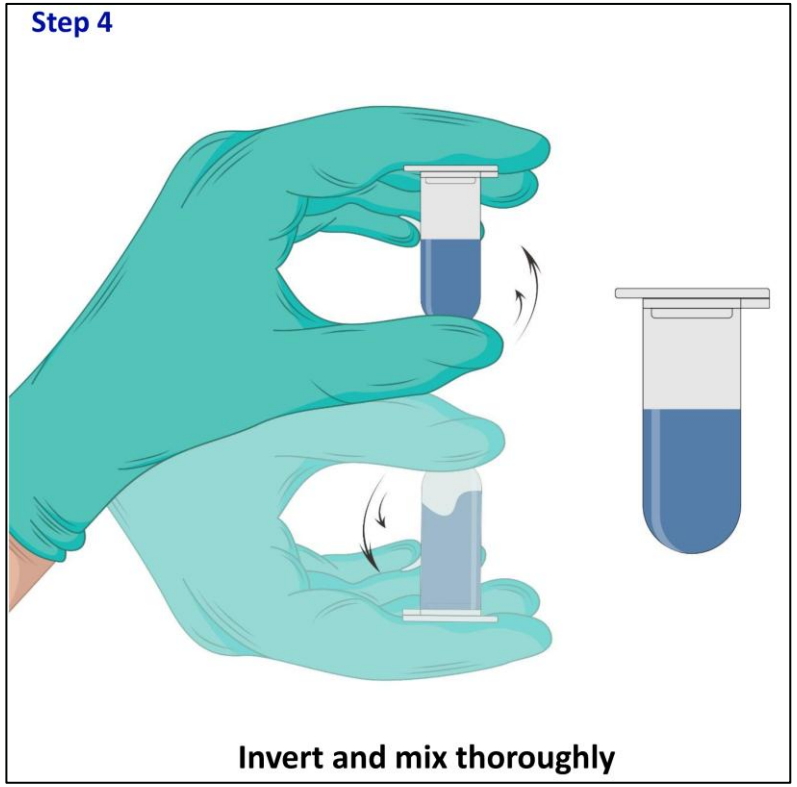
Sample Amount: Fill in a few millilitres in the empty tube. For example, if fill in the 3ml of empty tube for centrifugation, then fill 3ml in the empty space before centrifugation.

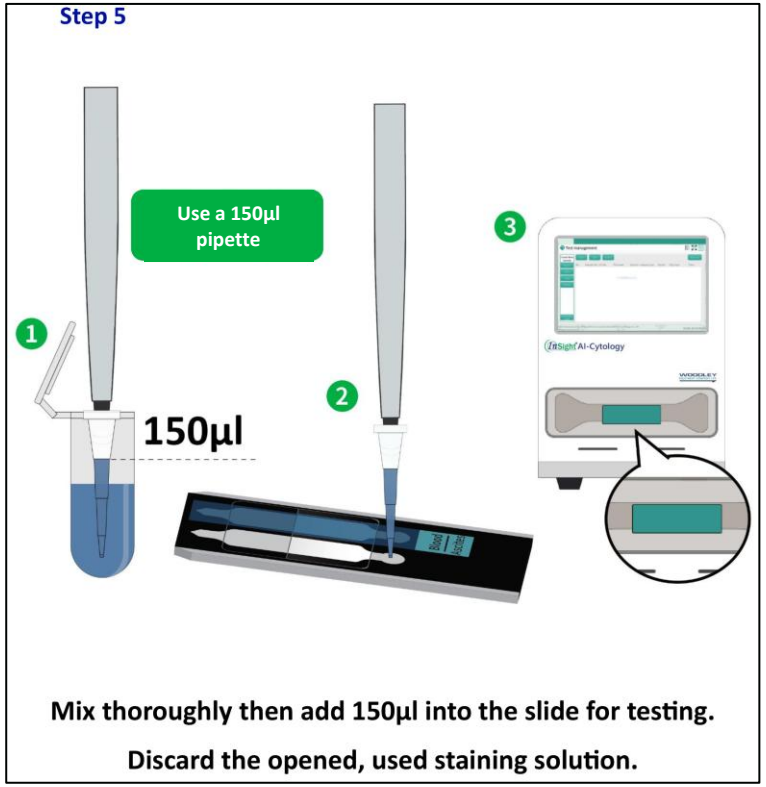
After centrifugation, the bottom 150 microliter sample was taken for detection.



Clear and Transparent Sample

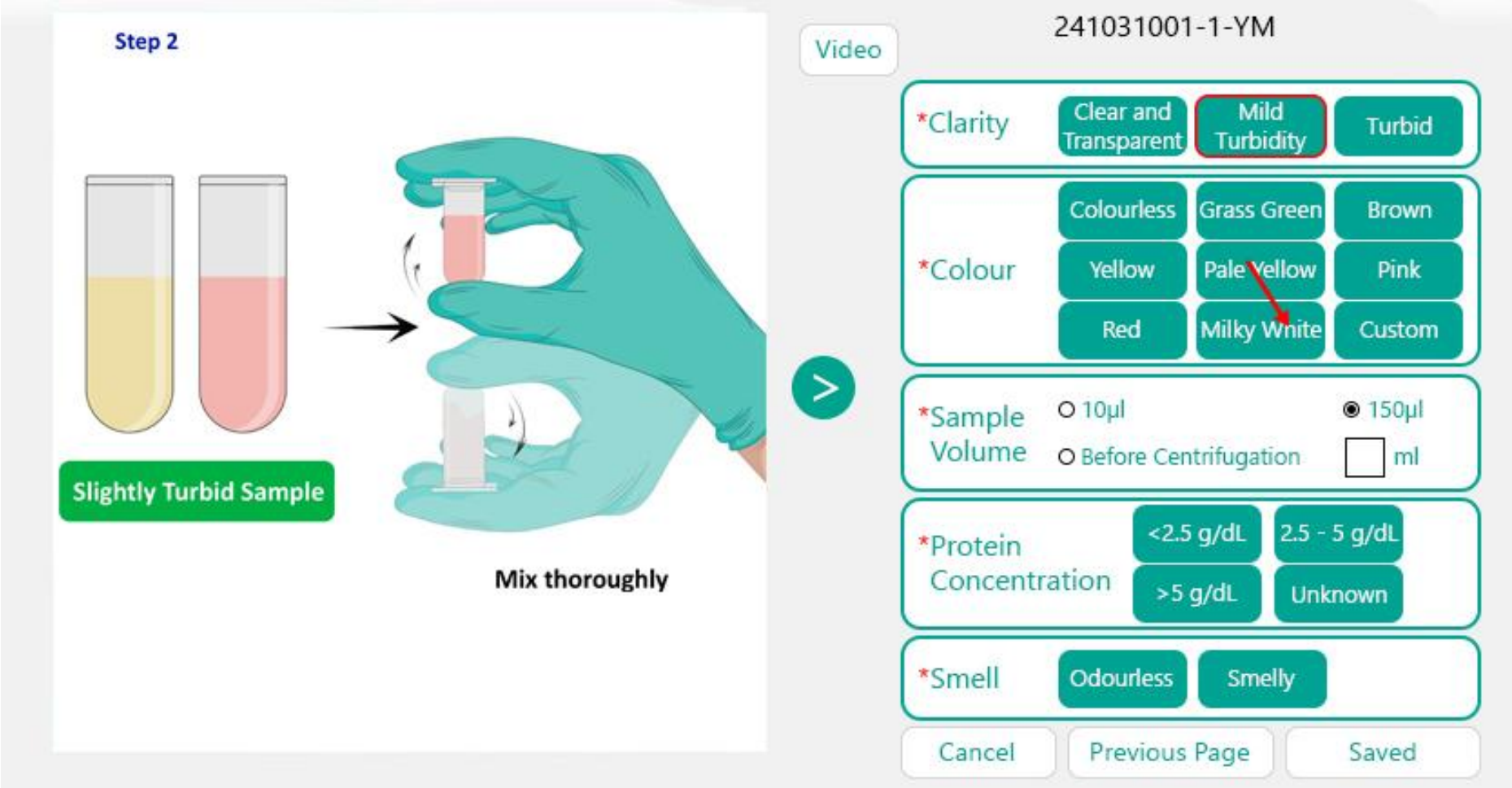






Mild Turbidity

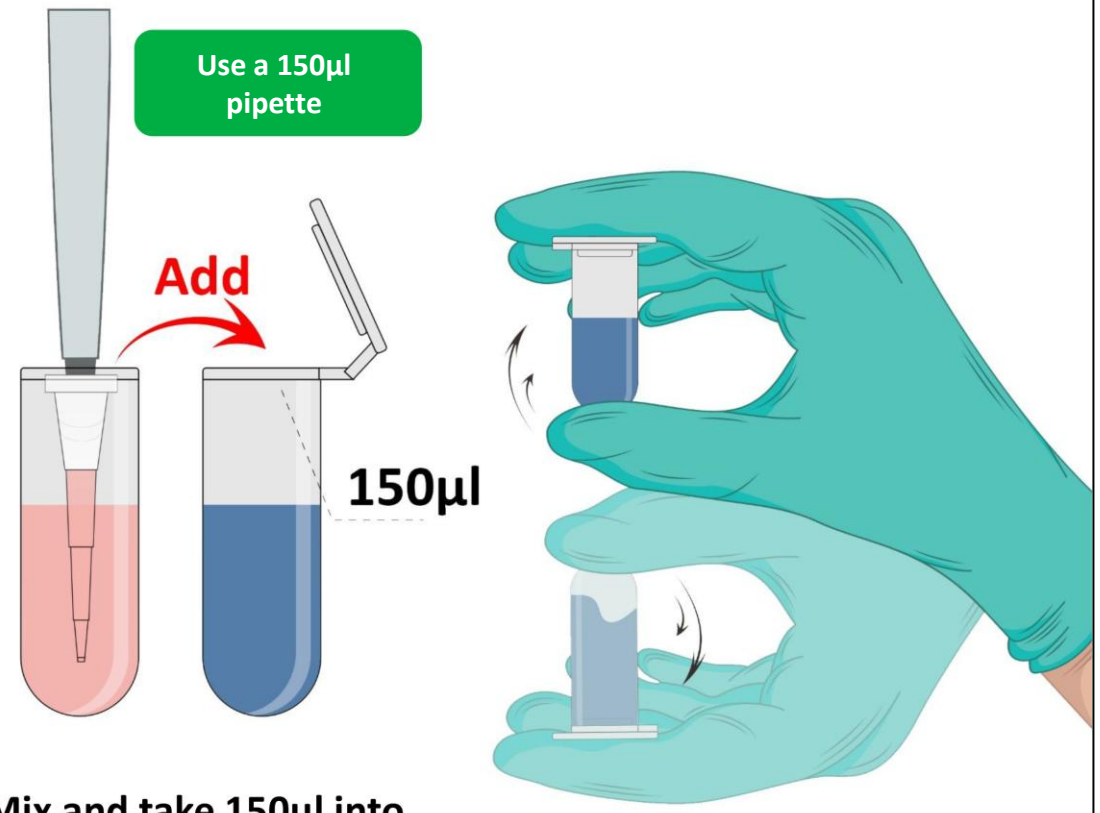
The mixture was directly sampled from 150µl for detection.



Mild Turbidity

Step 3

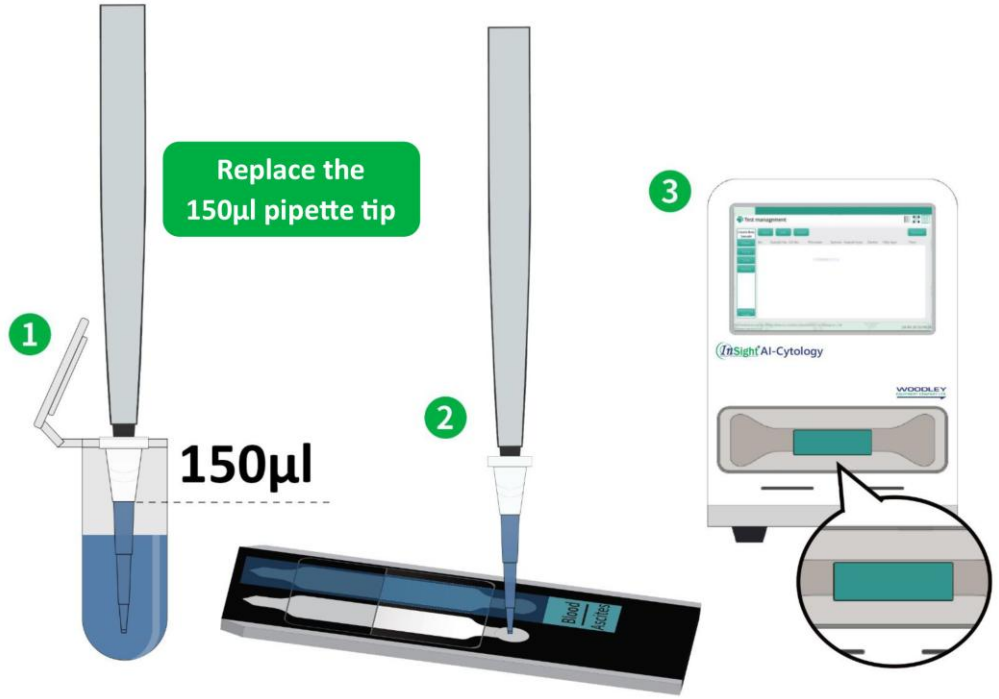
Use a 150µl pipette



Mix and take 150µl into the staining solution

Step 4

Replace the 150µl pipette tip

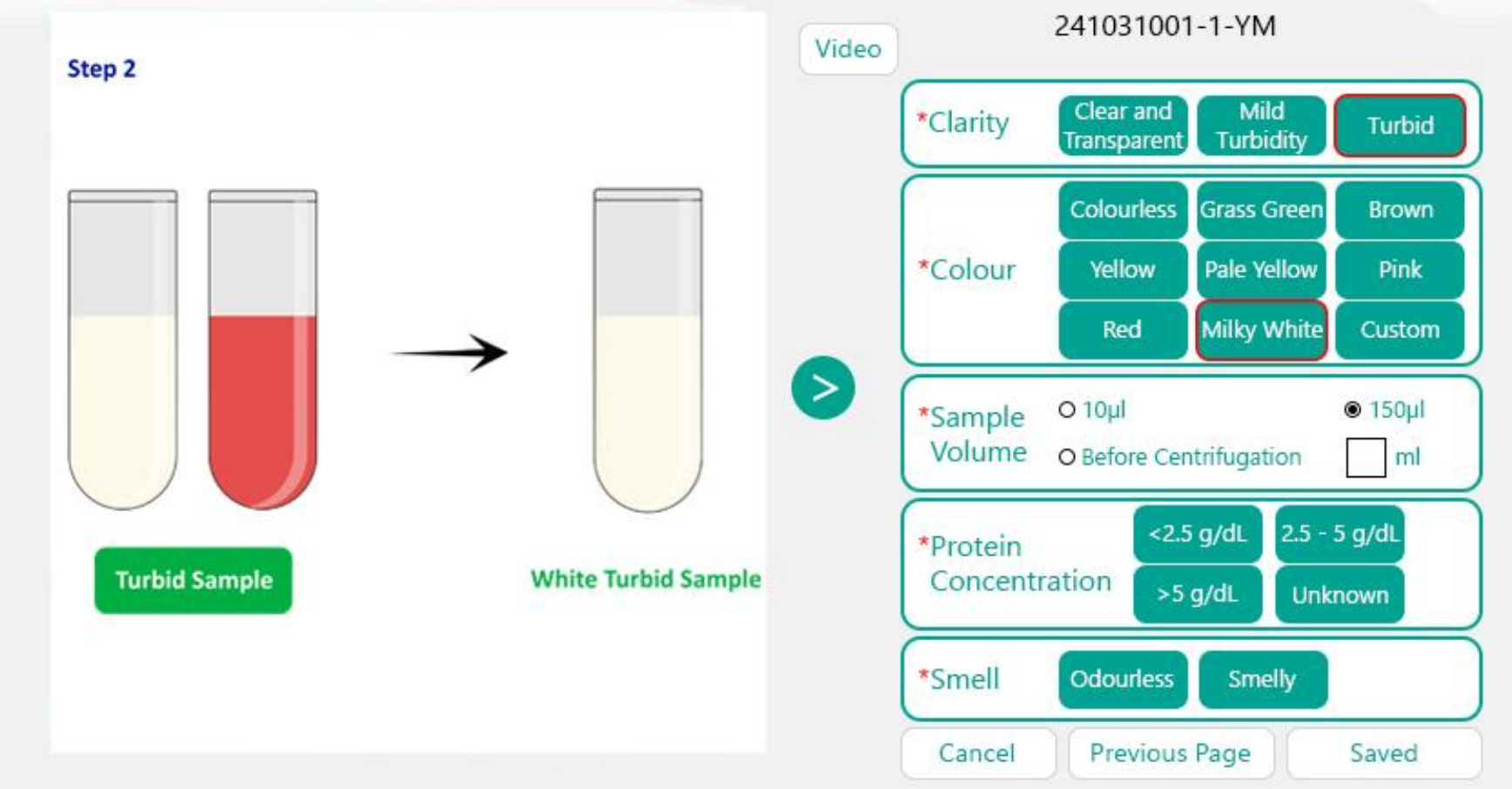


Mix thoroughly then add 150µl into the slide for testing.

Discard the opened, used staining solution.

White Turbid Sample

Special – The white turbid sample needs to be centrifuged first. The supernatant is taken to compare the turbidity to determine whether the sample amount is 10µl (purulent effusion) or 150µl (chylous effusion).



White Turbid Sample

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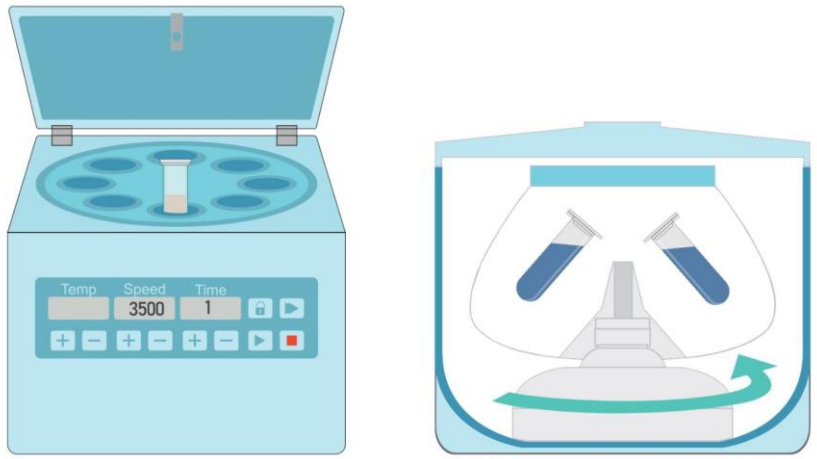
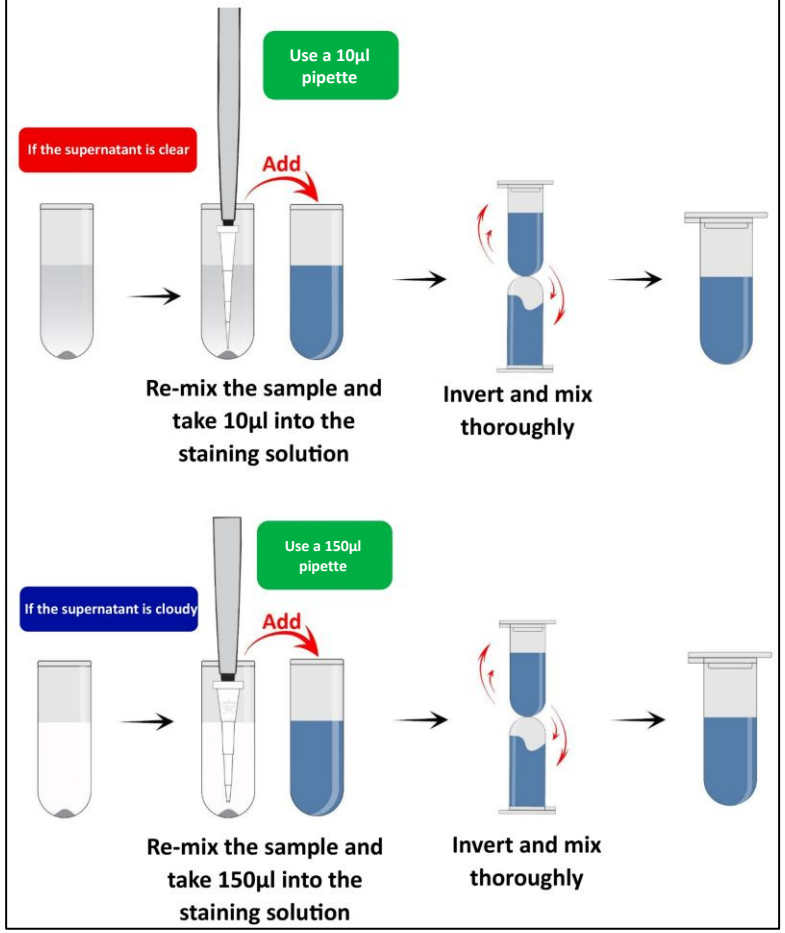
Step 3

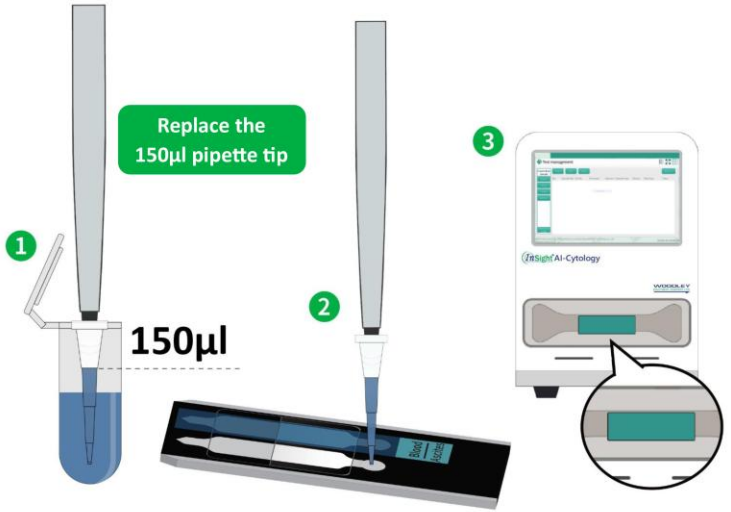


Centrifuge 2ml – 5ml at 3500 RPM for 5 minutes

Step 4



Step 5

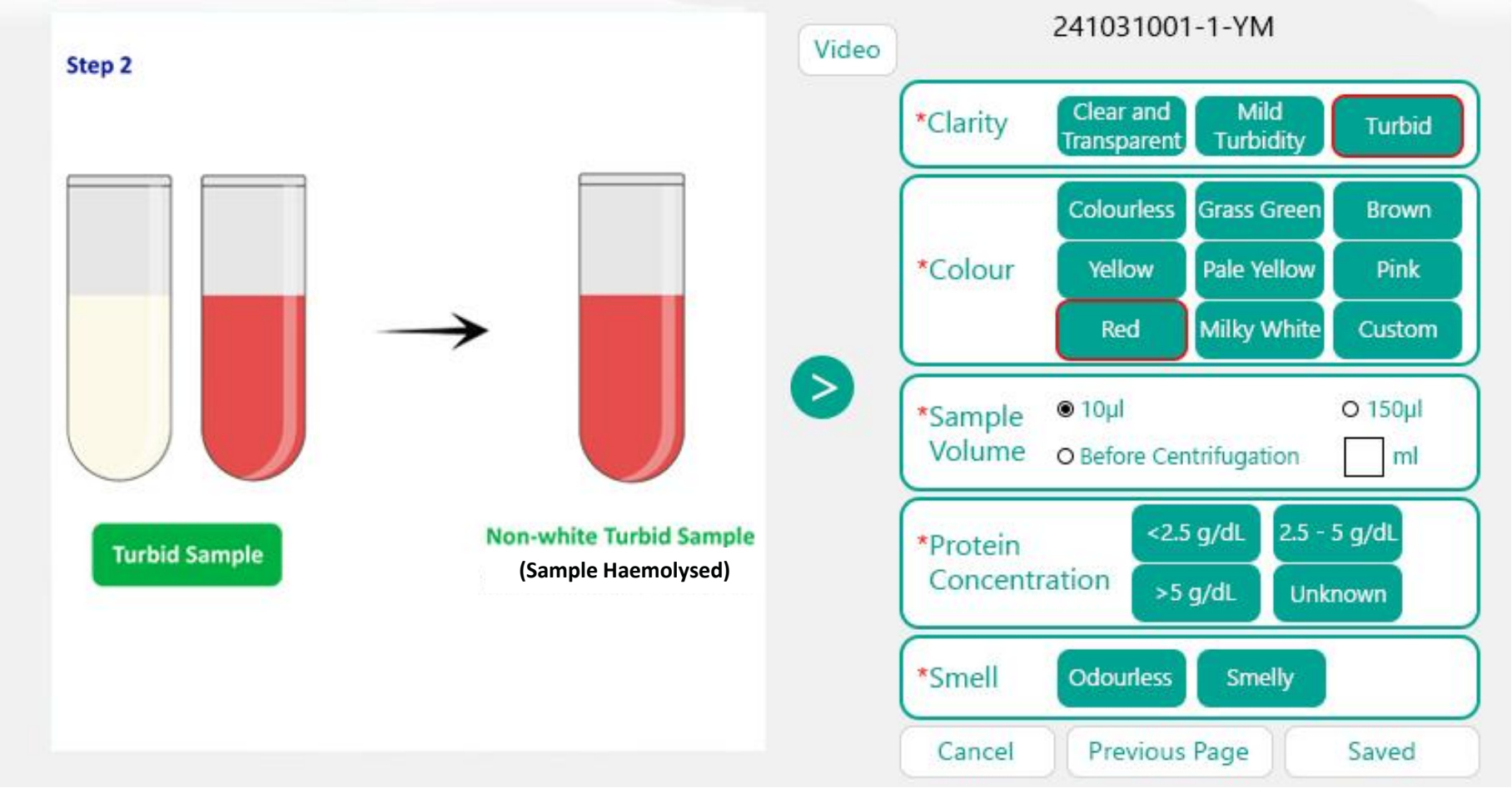


Mix thoroughly then add 150µl into the slide for testing.

Discard the opened, used staining solution.

Non-White Turbid Sample

For the non-white turbid sample, 10µl was added directly.



Non-White Turbid Sample

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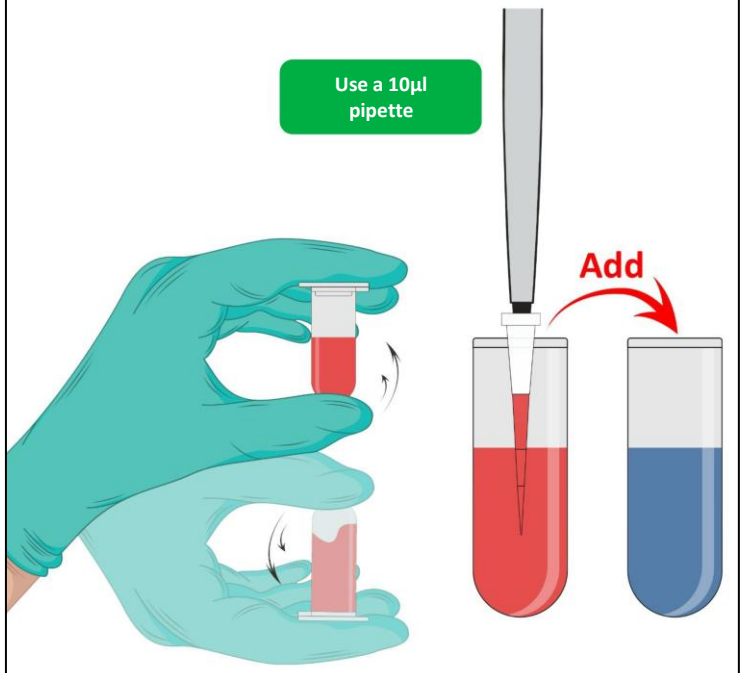
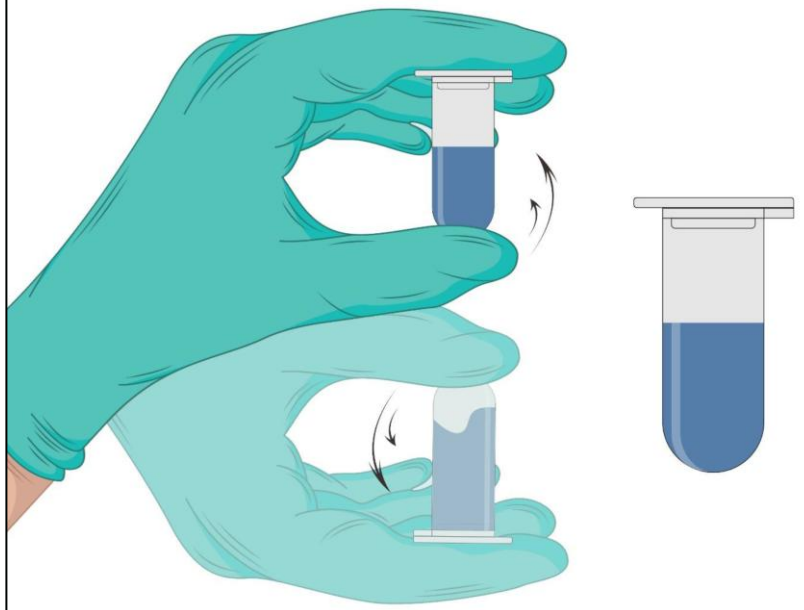
Step 3



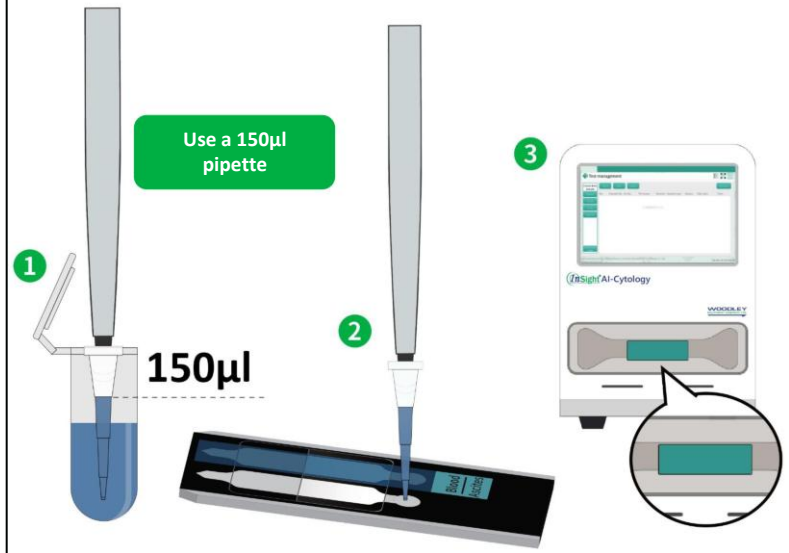
Mix and take 10µl into the staining solution

Step 4



Invert and mix thoroughly

Step 5



Mix thoroughly then add 150µl into the slide for testing.

Discard the opened, used staining solution.

Thank You