Pro-Slides™

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: PL.4960, PL.4961, PL.4962

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INTENDED USE

Pro-Slide™ Quality Control (QC) slides are intended for use in the quality control of staining techniques. Intended for laboratory use only.

SUMMARY AND EXPLANATION

Quality control is required by regulatory organisations. The use of QC slides with known reference materials has been well documented. Pro-Slides™ offer a reliable and traceable source of slide preparations for Gram staining, acid-fast staining, and Cryptosporidium staining techniques used routinely in microbiology.

PRINCIPLE OF THE TEST

Each Pro-Slide™ is clearly labelled with the application description, and contains fixed populations of the appropriate (non-viable) QC organisms for a negative and a positive control of the staining technique.

PL.4960	**Pro-Slide™ Acid-Fast Stain Control**	
	Positive	*Mycobacterium scrofulaceum* NCTC®10803 ATCC®19981*
	Negative	*Escherichia coli* NCTC®12241 ATCC®25922*
PL.4961	**Pro-Slide™ Gram Stain Control**	
	Positive	*Staphylococcus aureus* NCTC®12981 ATCC®25923*
	Negative	*Escherichia coli* NCTC®12241 ATCC®25922*
PL.4962	**Pro-Slide™ Cryptosporidium Stain Control**	
	Positive	Traceable culture of Cryptosporidium
	Negative	Acid-fast negative intestinal bacteria

MATERIALS PROVIDED

- PL.4960 Pro-Slide™ Acid-Fast Stain Control (50 Slides)
- PL.4961 Pro-Slide™ Gram Stain Control (50 Slides)
- PL.4962 Pro-Slide™ Cryptosporidium Stain Control (10 Slides)
- Instructions for use

MATERIALS REQUIRED BUT NOT PROVIDED

- Stains required for performing the staining technique (see range of Pro-Stains™)
- Microscope
- Immersion Oil PL.396

STABILITY AND STORAGE

Pro-Slides™ should be stored at 2°C-30°C in original container and avoid exposure to direct sunlight. Do not freeze. Product stored under these conditions will be stable until the expiry date shown on the product label.

PRECAUTIONS

- For *In Vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Do not use beyond the stated expiration dates.
- Microbial contamination may decrease the accuracy of the staining.
- Safety precautions should be taken in handling, processing and discarding all clinical specimens.
- Samples should be processed in the correct containment level conditions.
- Dispose of all materials in accordance with local regulations.
- Any serious incident that occurs in relation to the device should be reported to the manufacturer and the competent authority of the member state in which the incident occurred.

SAMPLE STORAGE AND COLLECTION

n/a

TEST PROCEDURE

Refer to your documented staining procedures or the IFU from the manufacturer of the stain. Examine using appropriate microscopy and record results as required by your QC compliance.
Pro-Lab Diagnostics manufacture a full range of microbiology stains (Pro-Stains™) and recommend the following staining methods.

GRAM STAINING (for PL.4961)

SUMMARY AND EXPLANATION / PRINCIPLE

The Gram stain was originally devised by Hans Christian Gram in 1884. The standard Gram staining method can be used to differentiate intact, morphologically similar bacteria into 2 groups: Gram positive bacteria have a thicker layer of peptidoglycan in their cell wall which retains the primary stain, appearing purple when finished, while Gram negative bacteria allow the primary stain to be flushed away due to a thinner peptidoglycan layer. This allows the final stain to counterstain the bacteria and giving a pink-red appearance when viewed under the microscope.

METHOD

1. Flood the slide with Crystal Violet or Methyl Violet, stand for 1 minute. Rinse with water.
2. Flood the slide with Grams or Lugols Iodine, stand for 1 minute. Rinse with water.
3. Gently decolourise with Grams Differentiator for approx. 10 seconds or Iodine Acetone for 1 minute. Rinse with water.
4. Flood the slide with counterstain, stand for 30 – 60 seconds.
5. Rinse well with water; gently blot dry.
6. Examine using a microscope.

INTERPRETATION OF RESULTS

Gram positive organisms – blue to purple.
Gram negative organisms – pink to red.

TB STAINING (for PL.4960)

SUMMARY AND EXPLANATION / PRINCIPLE

These methods are variations of the acid-fast method developed by Robert Koch in 1882. Mycobacteria possess unique acid-fast characteristics that make the acid-fast staining techniques invaluable in detecting Mycobacteria species.
The lipid content of the cell wall of acid-fast bacilli makes staining of the organisms difficult. If an organism is to be termed 'acid-fast', it must allow penetration of the stain, and resist decolourisation by acid alcohol. A counterstain is then used to emphasise the stained organism. The high concentration of phenol allows the stain to penetrate through the lipid layer, even after exposure to decolourisers.

METHODS

Ziehl-Neelsen Method

1. Flood the slide with ZN Carbol Fuchsin and heat gently (do not boil). Allow to stand for 10 minutes applying heat again after 5 minutes.
2. Rinse with water.
3. Flood the slide with Differentiator for ZN & Kinyoun Carbol Fuchsin for 10 minutes, applying a change of differentiator at 5 minutes.
4. Rinse with water.
5. Flood the slide with counterstain (Methylene Blue or Malachite Green), stand for 1 minute.
6. Rinse well with water; gently blot dry or dry using gentle heat.
7. Examine using a microscope.

Kinyoun Carbol Fuchsin Method

1. Flood the slide with Kinyoun Carbol Fuchsin, stand for 10 minutes.
2. Rinse with water.
3. Flood the slide with Differentiator for ZN & Kinyoun Carbol Fuchsin for 10 minutes, applying a change of differentiator at 5 minutes.
4. Rinse with water.
5. Flood the slide with counterstain (Methylene Blue or Malachite Green), stand for 1 minute.
6. Rinse well with water; gently blot dry or dry using gentle heat.
7. Examine using a microscope.

Auramine Phenol Method

1. Flood the slide with Auramine Phenol, stand for 10 minutes.
2. Rinse with water.
3. Flood the slide with differentiator for 10 minutes, applying a change of differentiator at 5 minutes.
4. Rinse with water.
5. Flood the slide with Potassium Permanganate or Thiazine Red, stand for 30 seconds.
6. Rinse well with water; gently blot dry or dry using gentle heat.
7. Examine using a fluorescent microscope.

INTERPRETATION OF RESULTS

Ziehl-Neelsen method: acid-fast bacilli are stained pink/red; other organisms are stained blue or green depending on the counterstain used.
Kinyoun carbol fuchsin method: acid-fast bacilli are stained pink/red; other organisms are stained blue or green dependent on the counterstain used.
Auramine phenol method: acid-fast bacilli appear as bright luminous rods against a dark background.

CRYPTOSPORIDIUM STAINING (for PL.4962)

SUMMARY AND EXPLANATION / PRINCIPLE

Cryptosporidium was first identified in 1976 and is one of the most common waterborne diseases found worldwide. Modifications of the acid-fast staining procedure can be used to identify *Cryptosporidium*; this method uses a high concentration of phenol to facilitate penetration of the dye into the cell wall of oocysts.
In the traditional *Cryptosporidium* method, cell wall components of *Cryptosporia* oocysts form a complex with carbol fuchsin which is retained in the cell wall after decolourisation. A counterstain is then used to emphasise the stained oocysts.
In the Auramine *Cryptosporidium* modification, both the outer and inner walls of *Cryptosporia* oocysts, as well as internal structures, are stained by the auramine O dye; the phenol component accelerates penetration through oocyst walls. The fluorescence is retained in the cell wall after decolourisation. A counterstain is then used to darken the background and other organisms, which emphasises the fluorescent oocysts.

METHODS

Cryptosporidium Method

1. Flood the slide with Cryptosporidium Stain, stand for 5 minutes.
2. Pour off excess stain and decolourise with Differentiator 1 until no more stain washes out of the smear. Rinse with water and shake off any excess.
3. Decolourise with Differentiator 2 for 2 minutes. Rinse with water and shake off any excess.
4. Flood the slide with Cryptosporidium Counterstain, stand for 1 minute.
5. Rinse well with water and gently blot dry, or dry using gentle heat.
6. Examine using a microscope.

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Revision: 2023 12

N.B. A single Cryptosporidium Differentiator may be used in place of Differentiator 1 and 2, replacing steps 2 and 3 with a single step: 'Decolourise with Differentiator for 2 minutes or until no more stain washes out of the smear.'

Cryptosporidium (Auramine) Method

1. Flood the slide with Auramine Phenol, stand for 10 minutes. Rinse with water.
2. Decolourise with Cryptosporidium differentiator (3% HCl) for 5 minutes. Rinse with water.
3. Flood the slide with Potassium Permanganate counterstain, stand for 30 seconds.
4. Rinse well with water. Allow to air dry or dry using gentle heat. Do not blot.
5. Examine using a fluorescent microscope.

INTERPRETATION OF RESULTS

Cryptosporidium method: acid-fast oocysts of *Cryptosporidia* are stained bright pink-red. Background material that is decolourised by differentiator will appear as pale green or pale red in colour.

Cryptosporidium (Auramine) method: *Cryptosporidia* oocysts will fluoresce bright green-yellow against a dark red background.

QUALITY CONTROL PROCEDURE

Quality control is carried out in accordance with recommended staining techniques for Pro-Stains™ manufactured by Pro-Lab Diagnostics; full details of the range are available on request from uksupport@pro-lab.co.uk.

LIMITATIONS OF THE PROCEDURE

- Only experienced personnel should carry out the interpretation of stained slides
- Read prepared slides as soon as possible after staining. Failure to do so may affect the results.

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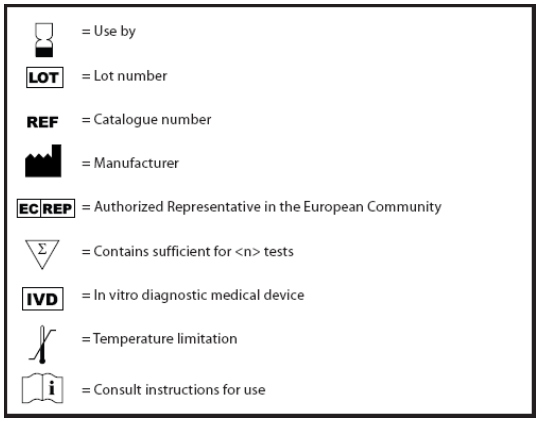

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HAZARDS IDENTIFICATION

	PL.4960 PL.4961 PL.4962	**This mixture is classified as not hazardous according to regulation (EC) 1272/2008 [CLP]. Not classified (SI 2019/758 as amended).**