PRO-SLIDES

(for *In Vitro* Diagnostic use only)

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

CE

INTENDED USE

Pro-Slide 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, TB staining, and Cryptosporidium staining techniques used routinely in microbiology.

PRINCIPLE

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
- Microscope
- Coverslip
- Immersion oil PL.396

STABILITY AND STORAGE

Pro-Slides should be stored at 2-30ºC 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 material in accordance with local regulations.

TEST PROCEDURE

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

GRAM STAINING (for PL.4961)

SUMMARY AND EXPLANATION

The Gram stain was originally devised by Christian Gram in 1884. The standard Gram staining method can be used to differentiate intact, morphologically similar bacteria into two groups. This is based on cell wall colour after employing the staining method. In addition, cell form, size and structural details are evident. This preliminary information can provide initial clues to the type of organism(s) present.

Gram postitve bacteria have a thicker layer of peptidoglycan in their cell wall which retains the primary stain, appearing purple when finished. Gram negative bacteria allow the primary stain to be flushed away due to a thinner peptidoglycan layer, leaving the final stain the chance to counterstain the bacteria and giving a 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 Gram's or Lugol's Iodine, stand for 1 minute. Rinse with water.
3. Gently decolorize with differentiator for approximately 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

These methods are variations of the acid-fast method developed by Robert Koch in 1882. Mycobacteria possess unique acid-fast characteritics 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 resist decolourization by acid-alcohol. A counterstain is then used to emphasise the stained organism.

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 and Kinyoun CF 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 and kinyoun CF 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 auramine 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 red, other organisms are stained blue or green dependent on the counterstain used.
Kinyoun carbol fuchsin method: Acid-Fast Bacilli are stained 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

Cryptosporidium was first identified in 1976 despite being one of the most common waterbourne diseases and is found worldwide. Modifications of the Acid-Fast staining procedure can be used to identify it and the method uses a high concentration of phenol to facilitate penetration of basic fuchsin dye into the cell wall of Cryptosporidium oocysts.

Cell wall components of Cryptosporia oocysts form a complex with carbol fuchsin which is retained in the cell wall after decolourization. Counterstain is then used to emphasise the stained oocysts.

METHODS

Modified Kinyoun Carbol Fuchsin Method

1. Place slide on a staining rack and fix in Cryptosporidium Fixative for 1 minute. Allow to air dry.
2. Apply Cryptosporidium stain to the slide and stain for 5 minutes.
3. Pour off excess stain and wash with differentiator 1. Rinse slide in water, and shake off any excess.
4. Apply differentiator 2 for 2 minutes or until no more stain washes out of the smear.
5. Wash off differentiator 2 by rinsing in water. Shake off excess water.
6. Apply Cryptosporidium counterstain and stain for 1 minute.
7. Remove excess stain by rinsing slide in water. Shake off excess water.
8. Blot slide gently on clean blotting paper and dry using gentle heat.
9. Examine using a microscope.

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Modified Auramine Phenol Method

1. Apply auramine phenol to the slide for 5 minutes.
2. Wash off excess stain by rinsing slide in water, shake off excess water.
3. Apply auramine differentiator for 10 minutes, applying a change of differentiator after 5 minutes.
4. Wash off differentiator by rinsing slide in water, shake off excess water.
5. Apply counterstain for 10 seconds.
6. Remove excess stain by rinsing slide in water, shake off excess water.
7. Blot slide gently on clean blotting paper, and dry using gentle heat.
8. Examine using a microscope.

INTERPRETATION OF RESULTS

Modified kinyoun carbol fuchsin method**:** acid fast oocysts of Cryptosporidia are stained bright red. Back-ground material that is decolourised by the differentiator 2 will appear as pale green or pale red in colour.

Modified auramine phenol method: acid fast oocysts of Cryptosporidia appear as bright luminous "doughnut" rings under fluorescent microscopy. The background that is decolourised by the differentiator solution and takes up the counterstain will appear as a dark colour.

QUALITY CONTROL PROCEDURE

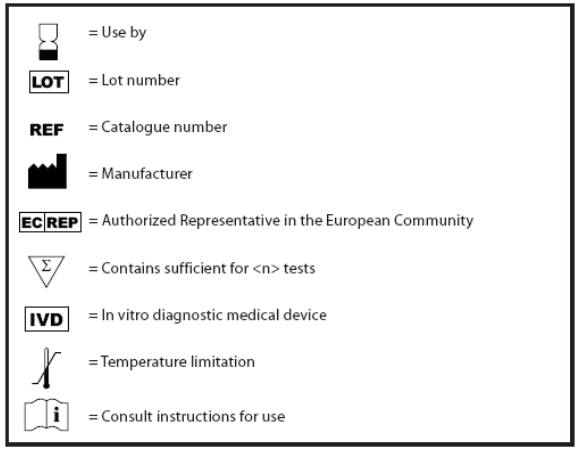
Quality control is carried out in accordance with recommended staining techniques for stains manufactured by Pro-Lab Diagnostics, full details of the range are available on request from **uksupport@pro-lab.com**

LIMITATIONS

- 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.

REFERENCES

- Anderson, N.L., et al, Cumitech 3B,; Quality Systems in the Clinical Microbiology Laboratory, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
- Arnold WM, Weaver RH. Quick microtechniques for the identification of cultures. Journal of Laboratory and Clinical Medicine 1948; 33:1334-7.
- Balzevic, D.J. and G.M. Edrer. (1975). Principles of Biochemical Tests in Diagnostic Microbiology. John Wiley & sons, New York, NY.
- Beveridge T,J Use of Gram stain in Microbiology.. Biotechnic and Histochemistry. 2001 May;76(3):111-8.
- Carmichael J.W. Lactofuchsin: a new medium for mounting fungi. Mycologia 1955; 4611.
- Chapin, K. C., and T.-L. Lauderdale. 2003. Reagents, stains, and media: bacteriology, p. 354-383.
- Cruickshank, R, J. P. Duguid, B. P. Marmion, R.H.A. Swain.The Practice of Medical Microbiology. 12th Edition. V2
- Gurr, E. A Practical Manual of Medical and Biological Staining Technique. 1953
- Hageage, G.J. B.J. Harrington, Use of calcofluor white in clinical mycology, Journal of Laboratory Medicne, 15, 109-12 (1984).
- Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol I Washington, DC: ASM, 1992.
- Jorgensen., et al. Manual of Clinical Microbiology, American Society for Microbiology, Washington, D.C.
- Kinyoun, J. J. 1915. A note on Uhlenhuth's method for sputum examination for tubercle bacilli. American Journal of Clinical Pathology. 46:472-4.
- Larone DH. Medically important fungi: a guide to identification. Washington DC: ASM Press, 1995.
- Lennette. Manual of Clinical Microbiology. Published by American Society for Microbiology, Washington, D.C., 1974
- Lowrance, B.L., P. Reich and W.H. Traub. (1969). Journal of Applied Microbiology 17:923-924.
- McInnis MR. Laboratory handbook of medical mycology. New York Academic Press, 1980.
- Monheit, J.E. D.F. Cowan, D.G. Moore, Rapid detection of fungi in tissues using calcofluor white and fluorescence microscopy, The Archives of Pathology and Laboratory Medicine. 108, 616-618 (1984).
- Murray, P. R. E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th edition. ASM Press, Washington, DC. 2003
- Nunns D, Mandal D, Farrand RJ, O'Neill H, Henshaw G. A comparison of acridine orange, wet microscopy and Gram staining in the diagnosis of bacterial vaginosis. Journal of Infection 1997; 34: 211-3.
- Payle B, Serrano L, Bieley HC, Reyes BA. Albert's solution versus potassium hydroxide solution in the diagnosis of tinea versicolor. International Journal of Dermatology. 1994 Mar;33(3):182-3.
- Sutter, V.L. and W.T. Carter. (1972). American Journal of Clinical Pathology 58:335-338
- Tille, P., et al. Bailey and Scott's Disgnostic Microbiology, C.V. Mosby Company, St Louis, MO.
- Wacko, R. and J.C. Sherris. (1963). American Journal of Clinical Pathology. 39:429-432.
- Ziehl, F. 1882. Zur Farbung des Tuberkelbacillus. Dtsch. Med. Wochenschr. 8:451.



EC REP

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HAZARD IDENTIFICATION

PL.4960 **PL.4961** **PL.4962**	Classification (EC 1272/2008)	NC Not Classified.

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