

## INTENDED USE

For the identification of *Trichomonas vaginalis* along with yeast cells in the diagnosis of bacterial vaginosis in prepared slides from clinical specimens.

## SUMMARY AND EXPLANATION

Acridine orange was first described in 1942 by Hilbrich and Strugger for the staining of microorganisms. It is now widely accepted for clinical use and used in many different areas.

## PRINCIPLE OF THE TEST

Acridine orange is a fluorochrome dye which differentially stains the nuclei of microorganisms. Acridine orange binds with the nucleic acid (DNA or RNA) present in microorganisms. At an acidic pH this fluoresces, helping in the differentiation of microorganisms and cellular components.

## MATERIALS PROVIDED

Concentrated Stains (dilute 1 part in 10 with saline, deionised or reverse osmosed water before use):

-	PL.8009	Acridine Orange	100 ml
-	PL.8009/4	Acridine Orange	400 ml
-	PL.8009/5	Acridine Orange	500 ml

Per 100ml solution:

- Acridine Orange contains 0.26g Acridine Orange powder.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Glass slides
- Inoculating loops
- Microscope
- Immersion Oil PL.396

## STABILITY AND STORAGE

Acridine Orange should be stored at 15°C-25°C in its original container and avoid exposure to direct sunlight. 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.
- Specimens should be processed in the correct containment level conditions.
- Dispose of all material in accordance with local regulations.

## TEST PROCEDURE

- Prepare a smear on a clean glass slide and allow to air dry.
- Stain the slide with Acridine Orange for 5-10 seconds.
- Wash the slide with tap water and decolourise with alcoholic saline\* for 5-10 seconds.
- Rinse with saline and allow to air dry.
- Add a drop of saline or distilled water to the smear and cover with a glass coverslip.
- Examine using a fluorescent microscope.

\*Alcoholic saline is made up of 2ml methanol + 98ml physiological saline.

## QUALITY CONTROL PROCEDURE

Internal quality control of Acridine Orange must be performed regularly on known reference material.

Recommended Quality Control:

Positive control – *Trichomonas vaginalis*

Negative control - A proven negative

## INTERPRETATION OF RESULTS

Positive- trophozoites of *Trichomonas vaginalis* are stained brick red with a yellowish-green banana shaped or rounder nucleus.










Negative- yeasts are stained red with a bright green nucleus and are significantly smaller and morphologically different. They are easily distinguishable from *Trichomonas*. Epithelial cells fluoresce yellow with a bright green nucleus. Leucocytes will only show slight yellow-green nuclear fluorescence.

## 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.

## REFERENCES

- Balzevic, D.J. and Edrer, G.M. (1975). Principles of Biochemical Tests in Diagnostic Microbiology. *John Wiley & sons*, New York, NY.
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- Murray, P. R., Baron, E. J., Jorgensen, J. H., Tenover, M. C. and Tenover, R. H. (ed.), Manual of clinical microbiology, 8th edition. *ASM Press*, Washington, DC. 2003
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- Tille, P. et al. Bailey and Scott's Diagnostic Microbiology, C.V. *Mosby Company*, St Louis, MO.

	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community
	= Contains sufficient for <n> tests
	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use

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## HAZARDS IDENTIFICATION

Please refer to Safety Data sheets for full text for all hazard and precautionary statements.

	PL.8009 PL.8009/4 PL.8009/5	Classification (EC 1272/2008) NC Not Classified.
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