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Directions For Use for the following:

Trichrome Stain Set for PVA (Zn) Fixative (Wheatley's Modification)

INTENDED USE
The Trichrome Stain Set for PVA (Zn) (Wheatley's Modification) is a complete set of reagents for use in performing the Wheatley's trichrome stain procedure on PVA (Zn) fixed fecal specimens for the identification of intestinal protozoa.

SUMMARY
Intestinal parasitic infections are diagnosed by recovery and identification of protozoan trophozoites and/or cysts or helminth eggs and/or juveniles. The detection and correct identification of intestinal protozoa is frequently dependent on the examination of a permanently stained smear as smaller protozoa are often missed with only the direct smear and concentration methods. The Trichrome stain has been used since 1929 as a histological stain for muscle tissue. In 1949, Gomori developed a shortened and rapid method for trichrome staining of histologic and cytologic sections. In 1951, Wheatley modified the Gomori procedure and, using Trichrome Stain, developed a rapid staining procedure for intestinal amoeba and flagellates.

FOR IN VITRO DIAGNOSTIC USE ONLY

PRECAUTIONS
This product should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers and media after their use. Directions should be read and followed carefully. Refer to the Safety Data Sheets for additional information.

STORAGE
Store the product in the original container at room temperature (15-30ºC). Avoid extremes of temperature and light. Keep all containers tightly closed when not in use.

USER QUALITY CONTROL
A positive parasite smear should be processed with each batch of slides or per the regulatory agency used by your laboratory to verify the quality of the staining reagents and the technique of the procedure. The product should not be used if the color has changed, the expiration date has passed or there are other signs of deterioration.

SPECIMEN COLLECTION AND PREPARATION
Permanent smears may be prepared from fresh or preserved fecal material. Specimens should be collected and handled following recommended guidelines.

Preparation Smears for Specimens Preserved In PVA (Zn) Fixative:
1. Place 1-2 drops of PVA (Zn) fixed specimen on a clean glass slide. Lay or hold the slide flat with the specimen side up. Using an applicator stick, gently and evenly spread the sample over the slide then, using a chopping motion, spread the specimen out to create thick and thin areas.
2. Lay the slide flat with the film up. Allow to dry at room temperature or 37°C for 3-24 hours.
3. Slides should be completely dry before staining.

PROCEDURE
Materials Provided:
The Trichrome Stain Set includes all reagents necessary to perform the Wheatley's Trichrome Stain procedure on PVA (Zn) fixed fecal specimens. All reagents are packaged in convenient, wide-mouth jars which can be used for the staining procedure. It is not necessary to transfer reagents to Coplin jars.

70% Ethanol	(2 x 50 ml)
Wheatley's Trichrome Stain	(1 x 50 ml)
Acid Ethanol, 90%	(2 x 50 ml)
100% Ethanol	(3 x 50 ml)
Xylene	(2 x 50 ml)

NOTE: Since the acid ethanol solution requires changing more frequently, the stain set is supplied with one replacement for Acid Ethanol, 90%.

Materials Not Provided:
Mounting medium, coverslips, immersion oil, microscope slides, applicator sticks, controls, absorbent paper.

STAINING PROCEDURE
1. Remove all jars from packaging box and line up sequentially, starting with "Container 1".
2. When ready to stain slide(s), remove caps from jars and place slide directly into the jar.
3. When finished, close lids tightly and store.

NOTE: Less contamination of reagents will occur if a blotting step is included between each staining step. Allow slides to drain between each solution.

Reagent	Timing
70% Ethanol (Container #1)	3-5 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
70% Ethanol (Container #2)	3-5 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
Wheatley's Trichrome Stain (Container #3)	10 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
Acid Ethanol, 90% (Container #4)	1-3 seconds. Immediately proceed to next step. Remove and drain off excess liquid by touching edge of slide to absorbent material.
100% Ethanol (Container #5)	5-10 seconds Remove and drain off excess liquid by touching edge of slide to absorbent material.
100% Ethanol (Container #6)	3 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
100% Ethanol (Container #7)	3 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
Xylene (Container #8)	10 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
Xylene (Container #9)	10 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material.
Coverslip and examine with oil immersion lens.	

NOTE: Staining time can vary depending on the intensity desired for the final stain result.

EXPECTED RESULTS
1. Typical staining reactions using Wheatley's Trichrome Stain Set with specimens adequately fixed in PVA (Zn) and stained are listed below:
2. The nuclear chromatin, chromatoid bodies, ingested erythrocytes and bacteria are purple to red-violet.
3. The cytoplasm of trophozoites and cysts stains blue-green tinted with purple.
4. Background material and artifacts stain blue-green to purple.
5. Helminth ova and larvae stain red to purple but may be more easily identified in a concentrated wet mount. .
6. Macrophages, leukocytes and yeasts show variable staining from green to blue to purple or red.
7. Special staining procedures are necessary for the identification of *Cryptosporidium, Cyclospora* and *Microsporidia*.

LIMITATIONS OF PROCEDURES
Wheatley's Trichrome Stain method is usually a trouble-free procedure when used as directed. Any problems that may occur will usually be one of the following:

Problem: Poor contrast of the chromatin material.
Cause: Over-decolorizing.
Solution: Decolorizing (acid ethanol, container #4) requires only a very brief contact, followed by an immediate dip in ethanol.

Problem: Poor staining of the cytoplasm and the nucleus. Degenerate forms that stain weakly.
Cause: Parasitic and cellular elements have degenerated because of improper fixation.
Solution: To ensure proper fixation, specimens must be placed in a fixative solution immediately after passage. The proportion of specimen to fixative (1:3) must be observed, be thoroughly mixed and have sufficient time for fixation.

Problem: Stained preparation is "cloudy" with poor contrast of cellular detail.
Cause: Carryover of solutions from one step to another. Excessive staining (more than 30-40 slides) will weaken or dilute the stain.
Solution: Change all solutions regularly to avoid staining clarity problems

Problem: Inadequate material for microscopic examination.
Cause: Smear is too thin.
Solution: Smears should be moderately thick. If necessary, concentrate the fixed specimen by centrifugation. Pour off excess specimen fixative to provide thicker material for smears.

Problem: Persistent staining failure after considering all of the above.
Cause: Faulty technique and/or contaminated reagents.
Solution: Discard the entire stain series. Try again with new staining solutions.

BIBLIOGRAPHY
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CONTACT
CalibreScientific US, Inc. offers a complete line of reagents, stains, and QC1™ Quality Control Slides for AFB, Parasitology, Bacteriology, and Mycology processing, as well as O&P collection systems and concentration devices for Parasitology. For Technical Assistance, email Technical@AlphaTecSystems.com, and for Customer Service, email Sales@AlphaTecSystems.com, or call either [+1] 800.221.6058 (USA) or [+1] 360.260.2779 between 8AM and 4PM Monday through Friday, Pacific Time.

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TRADEMARKS
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PRODUCT CODES:
0003125 Wheatley's Trichrome Stain Set, 1 Set (10 x 50 ml)

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GLOSSARY OF SYMBOLS

LOT	Batch code / Numéro de lot / Número de Lote / Numero di lotto / Lot Nummer / Lotnummer / Lotnummer / Šaržna številka / Número de lote
REF	Catalog number / Référence du catalogue / Número de catálogo / Numero di catalogo / Katalognummer / Catalog nummer / Het aantal van de catalogus / Kataloška številka / Número de catálogo
IVD	In vitro diagnostic medical device / Pour usage diagnostique in vitro / Para uso diagnóstico in vitro solamente / Solo per uso diagnostico in vitro / Nur zur Verwendung als in vitro-Diagnostikum / Alleen voor in vitro diagnostisch gebruik / För invitrodiagnostik enbart / Samo za invitro diagnostiko / Apenas para uso em diagnóstico in vitro
EC REP	Authorized representative in the European Community / Représentant européen autorisé / Representante Europeo Autorizado / Rappresentante europeo autorizzato / Autorisierter Europäischer Repräsentant / Germachtigde Europese vertegenwoordiger / Auktoriserad europeisk representant / Pooblaščen evropski predstavnik / Representante Europeu Autorizado
	Use-by date / Utiliser avant la date de péremption indiquée / Use antes de la fecha indicada / Utilizzare entro la data indicata / Bis zum angegebenen datum verbrauchen / Gebruik door vermelde datum / Använd innan angivet datum / Porabiti do navadenega datuma / Usar até à data indicada
	Manufacturer / Fabricant / Fabricante / Produttore / Hersteller / Fabrikant / Fabrikant / Proizvajalec / Fabricante
	Caution / Attention / Cuidado / Attenzione / Achtung / Voorzichtig / Iaktag försiktighet / Previdno / Atenção
	Temperature limit / Conserver aux températures indiquées / Almacene entre las temperaturas indicadas / Conservare a temperature comprese fra quelle indicate / Im angegebenen temperaturbereich aufbewahren / Opslaan bij een temperatuur tussen / Förvara mellan angivna temperaturer / Shranjevati med navedenimi temperaturami / Armazene entre as temperaturas indicadas
	Contains sufficient for <n> tests / Contenu suffisant pour <n> tests / Contiene suficiente para <n> pruebas / Contenuto sufficiente per <n> tests / Enthält ausreichend für <n> untersuchungen / Inhoud voldoende voor <n> testen / Innehåller tillräckligt för <n> tester / Vsebina zadostuje za <n> testov / Contém quantidade suficiente para <n> testes
	Consult instructions for use / Consulter la notice d'utilisation / Consulte las instrucciones para el uso / Consultare le istruzioni per l'uso / Bitte beachten Sie die Anwendungsvorschriften / Raadpleeg instructies voor gebruik / Konsultera bruksanvisningen innan användning / Glej navodila za uporabo / Consulte instruções para o uso
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