INTENDED USE
The Trichrome-PLUS™ Stain Set is a complete set of reagents for use in the identification of intestinal protozoa in fecal specimens preserved in PVA (Zn).

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. In comparison to the Wheatley’s Trichrome procedure, the Trichrome-PLUS Stain offers these advantages: shorter staining time, fewer staining reagents and steps, and the cytoplasm usually stains more distinctly which aids in identification.

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 product in the original container at room temperature (15-30°C). 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 of smears for staining specimens preserved in PVA (ZN):
Place 1 to 2 drops of the PVA (Zn) fixed specimen onto a clean microscope slide. Using an applicator stick, cover one-third of the slide surface, spreading the specimen to the edge of the slide. Use the applicator stick and “chop” the specimen, spreading it out to create thick and thin areas. Lay flat with the film up. Allow to dry thoroughly at room temperature or 37°C for 3-24 hours. Slides should be stained when completely dry.

PROCEDURE
Materials Provided:
The Trichrome-PLUS Stain Set includes all reagents necessary to perform the Trichrome-PLUS staining procedure:
70% Ethanol (2 x 50 ml)
100% Ethanol (4 x 50 ml)
Trichrome-PLUS Stain (1 x 50 ml)
Trichrome-PLUS Decolorizer (2 x 50 ml)
PRO-CLEAR™ (2 x 50 ml)

NOTE: Since some solutions require changing more frequently, the Trichrome-PLUS Stain set is supplied with the following replacements: 70% Ethanol (1), Trichrome-PLUS Decolorizer (1), 100% Ethanol (1).

Materials Not Provided:
Mounting Medium, Coverslips, Microscope Slides, Immersion Oil, Absorbent Paper, Coplin Jars, Distilled Water

TRICHROME-PLUS STAINING PROCEDURE

Reagents | Timing |
--- | --- |
70% Ethanol (Container #1) | 1 minute. Remove and drain off excess liquid by touching edge of slide to absorbent material. |
Distilled water (Container #2) | 5 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material. |
Trichrome-PLUS Stain (Container #3) | 11 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material. |
Distilled water (Container #4) | 5 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material. |
Trichrome-PLUS Decolorizer (Container #5) | 1 dip (1-2 seconds). Remove and drain off excess liquid by touching edge of slide to absorbent material. |
100% Ethanol (Container #6) | 10 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material. |
100% Ethanol (Container #7) | 10 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material. |
100% Ethanol (Container #8) | 1 minute. Remove and drain off excess liquid by touching edge of slide to absorbent material. |
PRO-CLEAR (Container #9) | 30 seconds. Remove and drain off excess liquid by touching edge of slide to absorbent material. |
PRO-CLEAR (Container #10) | 3 minutes. Remove and drain off excess liquid by touching edge of slide to absorbent material. |

Mount coverslip and examine under oil immersion lens.

NOTE: For ease of use, prepare two Coplin jars of distilled water. Label one jar as “Container 2” and the other “Container 4”.

EXPECTED RESULTS
1. Typical staining reactions using Trichrome-PLUS Stain Set with adequately fixed and stained PVA (Zn) slides are as follows:
2. The nuclear chromatin, chromatoid bodies, ingested erythrocytes and bacteria stain red to red-violet.
3. The cytoplasm of trophozoites and cysts stains blue to blue-violet.
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.

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Macrophages, leukocytes and yeasts show variable staining from green to blue to purple or red.

Special staining procedures are necessary for the identification of Cryptosporidium, Cyclospora and Microsporidia.
Directions For Use for the following:

**Trichrome-PLUS™ Stain Set for PVA (Zn) Fixative**

**LIMITATIONS OF PROCEDURES**

The Trichrome-PLUS staining 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 requires only a very brief contact, followed by an immediate dip in ethanol.

**Problem:** The smear is too green or exhibits 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 staining or decolorizing 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 stain set and clean glassware.

**BIBLIOGRAPHY**


**CONTACT**

Alpha-Tec Systems, 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.

**WARRANTY**

This product is warranted by Alpha-Tec Systems, Inc. to perform as described in the labeling and literature supplied. Alpha-Tec Systems, Inc. disclaims any implied warranty or merchantability or fitness for any other purpose, and in no event shall Alpha-Tec Systems, Inc. be liable for any consequential damages arising out of aforesaid express warranty.

**TRADEMARKS:**

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**PRODUCT CODES**

0004119     Trichrome-PLUS Stain Set, 1 set, 11 x 50 ml
Directions For Use for the following:

Trichrome-PLUS™ Stain Set for PVA (Zn) Fixative

Manufactured by Alpha-Tec Systems, Inc.
1311 SE Cardinal Court, Suite 170
Vancouver, WA 98683 USA

Glossary of Symbols

Lot
- Batch code / Numéro de lot / Número de Lote / Numero di
  lotto / Löt Nummer / Lotnummer / Lotnumer / 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 invito diagnostiko /
  Apenas para uso em diagnóstico in vitro

EC/REP
- Authorized representative in the European Community / Représentant européen autorisé / Representante Europeo
  Autorizado / Rapresentante europeo autorizzato / Autorisierter
  Europäischer Repräsentant / Gemachtidge
  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 a data indicada

Manufacturer / Fabricant / Fabricante / Produttor / Hersteller
/ Fabrikant / Fabrikant / Proizvajalec / Fabricante

Caution / Attention / Cuidado / Attenzione / Achtung / Voorzichtig / laktak
forsikthet / Previdno / Atenção

Temperature limit / Conserver aux températures indicées / Almacene
entre las temperaturas indicadas / Conservare a
temperatura comprese fra quelle indicate / Im angegebenen
temperaturbereich aufbewahren / Oplaan bij een
temperatuur tussen / Förvara mellan angivna temperaturer /
Shranjevali med navedenimi temperaturami / Armazen entre
as temperaturas indicadas

Contains sufficient for <n> tests / Contenu suffisant pour <n>
tests / Contiene suficiente para <n> pruebas / Contenido
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 / Giej
navodila za uporabo / Consulte instruções para o uso