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Phytohaemagglutinin (Reagent Grade)

INTENDED USE

Phytohaemagglutinin is used to stimulate mitotic division of lymphocytes maintained in a cell culture system and facilitate cytogenetic studies of the chromosomes.

SUMMARY AND EXPLANATION OF THE TEST^{3,5,6}

Phytohaemagglutinin (PHA) is the generic name given to aqueous extracts of the seeds of certain plants, notably *Phaseolus* spp. Originally used for separating leucocytes from whole blood, on account of its powerful erythroagglutinating property², it was later found to cause progressive mitotic division of lymphocytes in tissue culture⁴.

Subsequent experience has shown that PHA is the most universally effective and convenient stimulant of lymphocytes from many animal species. The widespread popularity of peripheral blood culture as a means of chromosome analysis has been largely dependent on this property.

PRINCIPLE OF THE PROCEDURE

The procedure involves establishing a suitable lymphocyte culture system to which PHA is added. This stimulates mitosis which is arrested in metaphase by the addition of colcemid (deacetyl methylcolchicine). At this stage the chromosomes are most clearly visible. The sample is treated with hypotonic solution which results in swelling of cells and erythrocyte lysis facilitating chromosome identification in suitably fixed and stained slide preparations.

REAGENT	
KIT CONTENTS	
Phytohaemagglutinin (Reagent Grade)	
HA15/R30852701	1 bottle

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**.



The freeze dried material will retain its reactivity at least until the date shown on the bottle label when stored at 2 to 8°C.

PHYTO-HAEMAGGLUTININ

Phytohaemagglutinin (reagent grade)

A freeze dried preparation of an aqueous extract from selected seed beans of *Phaseolus* spp. Each bottle contains approximately 45 mg of freeze dried extract.Each bottle should be reconstituted by the addition of 5 ml of sterile physiological saline using a sterile disposable hypodermic syringe. Note that the use of other solutions may cause cloudiness. The cap of the bottle should be sterilised by wiping with ether, the needle should pierce the centre of the stopper and be held in a vertical position during reconstitution.

Reconstituted material should be stored at 2 to 8°C and used within one month.

WARNINGS AND PRECAUTIONS

For professional use only.

Please refer to the manufacturer’s safety data sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

- Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for at least 15 minutes at 121°C. Disposables should be autoclaved or incinerated.
- Spillage of potentially infectious material should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a standard bacterial disinfectant or 70% alcohol. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.
- Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- In accordance with the principles of Good Laboratory Practice it is strongly recommended that samples should be treated as potentially infectious and handled with all necessary precautions.

ANALYTICAL PRECAUTIONS

- Material showing bacterial contamination should be discarded; slight turbidity sometimes develops in the absence of bacterial growth and has no deleterious effect on the properties of the PHA.
- Aseptic techniques are essential.
- Do not use reagent beyond the stated expiry date.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

SPECIMEN COLLECTION⁶

The success of whole blood cultures for cytogenetic analysis is influenced by the level of lymphocytes with normal function at the time of sampling. As this level can be affected by infection and drugs, wherever possible subjects for cytogenetic studies should not have taken drugs for 7 days prior to collection of blood for tests. Similarly, the mitotic index may be greatly reduced during the anergic phases of certain diseases (e.g. Hodgkin’s disease, sarcoidosis, etc.) and, to a lesser degree, in normal individuals during the later stages of pregnancy.

Preservative must not be added to blood samples for lymphocyte culture. Aseptic techniques are essential.

SPECIMEN STORAGE

Blood samples should be tested without delay wherever possible. If absolutely necessary, they may be stored at 2 to 8°C for no longer than 48 hours.

PROCEDURE

MATERIALS PROVIDED

See **Kit Contents**.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Microculture medium
 - Sufficient for 25 tests
 - 100 ml RPMI 1640 medium
 - 25 ml Foetal calf serum
 - 1.25 ml Glutamine (200 mM)
 - 1.25 ml Penicillin (5000 IU/ml)/Streptomycin (5000 µg/ml)
 - 2.5 ml Phenol-free heparin (1000 IU/ml)
- Colcemid (Sigma Laboratories) 25 µg/ml solution.
- 75 mM potassium chloride solution.
- Acetic alcohol. 1 part glacial acetic acid: 3 parts methanol (analytical reagent grade).
- Giemsa or 2% acetic acid-orcein.
- Mountant.
- Glass slides and coverslips.
- Plastic centrifuge tubes.
- Incubator.
- Bench centrifuge.
- Vortex Mixer.
- Light microscope.

TEST PROCEDURE^{1,6}

Whole blood or separated leucocytes have been used for cytogenetic studies, but the former is the simplest and most widely used in routine studies. As with any cell culture procedure, optimal results are dependent on establishing adequate culture conditions. Serum additives should be screened prior to use to ensure the selection of batches free of inhibitory effects. Since the relative content of active PHA may vary slightly between different batches, it may be beneficial to test two concentrations of PHA.

- A.**

Preparation of whole blood microcultures
- Step 1**

Collect 5 to 20 ml of fresh blood in phenol-free heparin and mix by inversion.
- Step 2**

Reconstitute Phytohaemagglutinin by adding 5 ml sterile physiological saline using a sterile syringe.
- Step 3**

Using aseptic technique, prepare required volume of microculture medium allowing 5 ml for each blood sample.
- Step 4**

Dispense microculture medium to appropriately labelled sterile screw-capped bottles and aseptically add 0.1 ml reconstituted PHA. Immediately before culture, add 0.4 ml of heparinised blood using a sterile disposable syringe.
- Step 5**

Incubate the cultures at 37°C for 72 hours. Mix each bottle by inversion daily.

- B.**

Harvesting the Cultures
- Step 1**

Remove culture bottles from the incubator 1.5 hours prior to harvesting.
- Step 2**

Add 0.15 ml of a solution of Colcemid (25 µg/ml) to each culture.
- Step 3**

Mix gently and return to the 37°C incubator.
- Step 4**

Remove the cultures from the incubator and transfer to plastic graduated centrifuge tubes marked with sample details.
- Step 5**

Centrifuge the cultures in a bench centrifuge at 500 g for 5 minutes.
- Step 6**

Remove most of the supernatant fluid and discard.
- Step 7**

Resuspend the deposit in 6 to 8 ml of 75 mM potassium chloride solution pre-warmed to 37°C and incubate at 37°C for 10 minutes.
- Step 8**

Centrifuge as in 5 and discard supernatant.
- Step 9**

Using a Pasteur pipette slowly add 6 to 8 ml of freshly prepared acetic alcohol to the deposit whilst agitating constantly on a Vortex mixer. Add the fixative dropwise at first, followed by a slow trickle to minimise cellular damage and formation of lumps.
- Step 10**

Leave at 2 to 8°C for 10 minutes.
- Step 11**

Centrifuge lightly, remove the supernatant fluid as before and slowly add a further 5 ml of acetic alcohol to resuspend the deposit.
- Step 12**

Repeat step 11 twice more, re-suspending finally in 0.5 ml acetic alcohol. Use this cell suspension to prepare slides for examination. Care must be taken to avoid agitation of the cells.
- C.**

Preparation of slides
- Step 1**

Slides must be scrupulously clean. A suitable cleaning procedure is to soak the slides in chromic acid overnight, after which they should be washed in running water for at least half an hour and polished with a glass cloth.
- Step 2**

Apply 1 or 2 drops of the re-suspended cell preparation to the centre of a glass slide and allow to spread.
- Step 3**

Wipe excess fixative from the edges of the slide with filter paper.
- Step 4**

At the first appearance of Newton’s Rings, blow gently to speed final drying of the slide.
- Step 5**

Stain with Giemsa or 2% acetic acid-orcein stain and mount.

Fixed, stained and mounted slides have an indefinite life if correctly stored. Low power scanning, high power observation and subsequent photomicrography ultimately lead to the preparation of karyograms which can be studied in detail and filed with case reports.

RESULTS

QUALITY CONTROL

Several factors including source of sample, culture conditions and selection of reagents can influence the result obtained. Users are recommended to run each new batch of reagent in parallel with reference material of known, suitable activity before adoption in routine use.

LIMITATIONS OF THE PROCEDURE

The precise mechanism of lymphocyte transformation is not yet fully understood and unexplained technical failures sometimes occur. Serum factors, as yet unidentified, can cause interference but microbial contamination of the cell culture system remains the most frequent cause of failure. Hence, scrupulous attention to details of technique has to be given to lymphocyte culture systems if they are to be regularly successful. Optimal results are reliant on both the selection of culture conditions and reagents which support a good mitotic index. Hence screening of serum additives prior to use, titration of PHA and a knowledge of the possible effect of patient history on lymphocyte response may be beneficial.

CLINICAL APPLICATIONS

The modal human chromosome number is 46. Human chromosomes have been classified according to their length and the position of the centromere (Denver classification⁷). Aberrations of chromosomal constitution have been associated with a number of congenital disorders such as Down’s Syndrome (typically with an additional small autosome) and syndromes associated with indeterminate sexuality (Turner’s syndrome, Klinefelter’s syndrome and others, where the sex chromosomes are found to be abnormal). An acquired chromosomal abnormality can be detected in a proportion of the leucocytes in chronic myelocytic leukaemia (the “Philadelphia” chromosome) and the progress of treatment may be assessed by following this marker. As the limit of tolerance is approached in radiation therapy, there is a marked increase in the proportion of cells with bizarre chromosomal constitution and the appearance of these cells has been used as a guide to dosage.

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⁶ **Watt, J.L. and Stephen, G.S.** (1986). Lymphocyte culture for chromosome analysis. *In* Human Cytogenetics: a practical approach, pp. 39-55. Eds. D.E. Rooney and B.H. Czepulkowski, IRL Press Ltd., Oxford.

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PACKAGING

	HA15/R30852701.....5 ml
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Symbol legend

	Catalog Number
	Consult instruction for use (IFU)
	Temperature limitation (Storage Temp.)
	Batch code (Lot Number)
	Use by (Expiration Date)

	Manufacturer
IFU X7813, Revised November 2011 Printed in the UK	

	Remel Europe Ltd Clipper Boulevard West, Crossways Dartford, Kent, DA2 6PT UK
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