Processing of Chorionic Villus Samples with Anti-Clotting Agent  
(Genial Genetics Solutions Ltd)

This procedure is only suitable for samples that do not require direct karyotyping. Use of Anti-Clotting Reagent results in the breakdown of trophoblast cell layers, which may compromise direct karyotyping.

Anti-Clotting Reagent (ACR) (Genial Genetic Solutions Ltd)

200mg (one vial) of ACR powder dissolved in 100mls of phosphate buffered saline (PBS). Filter sterilise with a 0.22 µm syringe filter and aliquot into 5ml lots. Store at -20°C and thaw as required.

Method

1. Pour tissue from sample container into a large petri dish.
2. Remove and discard any transport media.
3. Add 5mls of ACR solution (2mg/ml) to the sample.
4. Observe sample (by eye) every 5 to 10 minutes watching for dissociation of blood clots from the tissue. The breakdown of the clot can be facilitated by gentle agitation of the tissue. Samples usually require between 10 and 30 minutes treatment in ACR, but some extremely clotted samples may require longer.
5. Transfer tissue to a clean petri dish and add 10mls of sterile PBS to sample.
6. Rinse sample and remove and discard PBS.
7. Add 10mls of fresh PBS to remove any remaining blood in solution.
8. Repeat until tissue and PBS are "clean".
9. Assess cleared sample for the presence of fetal material.
10. Tissue is now cleared to establish cultures according to standard procedures.

Setting Up of CVS Cultures Using the Collagenase Dissociation Method

Media And Reagents

- Trypsin/EDTA  Dissolve 0.2g Trypsin (1:250 activity) and 0.08g EDTA in 400ml saline. Filter sterilise before use using 0.22µm syringe filter.
- Collagenase Culture Media (CCM) 50ml RPMI 1640 and 50ml Hams F10. 1ml Penicillin/Streptomycin (10000 IU/ml penicillin;10000 UG/ml streptomycin).
- Collagenase  2mg/ml of collagenase (type IV) in CCM. This can be stored at -20°C until required.

Method

1. Transfer tissue into a culture tube containing 4ml of sterile Trypsin/EDTA and incubate at 37°C for 1 hour, mixing the tube occasionally.
2. Allow the tissue to gravity settle and then, using a sterile pastette, carefully remove the Trypsin/EDTA (which contains most of the cytotrophoblast cells) and discard.
3. Using a 0.22 µm syringe filter, sterilise 1ml of CCM and 2ml of collagenase solution and add to the pellet. Mix well.
4. Incubate for approximately 2 hours at 37°C with occasional mixing until tissue has broken down to a suspension.
5. Centrifuge at 340g for 5mins.
6. Carefully remove the supernatant and discard.
7. Divide pellet of cells between culture tubes/dishes as appropriate and feed with culture medium.
8. Incubate at 37°C for 2-3 days before checking for settling and growth.
A Novel Technique for the Removal of Blood Clots from Chorionic Villus Samples Using Anti Clotting Reagent

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Introduction
Diagnostic Chorionic Villus Samples (CVS) are often submitted for karyotyping with varying degrees of blood clot attached. Routinely the method of removal of the blood is dissection using needles. This is necessary as maternal cell contamination (MCC) is often masked within these clots, however the use of needles to sort this biological material poses health and safety risks. Previous attempts to identify an alternate method of sample sorting have proved unsuccessful. Anti Clotting Reagent (ACR)[Genial Genetic Solutions Ltd], is a newly available product for use on clotted peripheral blood samples prior to culture for chromosome analysis.

Aim
The aim of this study was to investigate whether ACR was a suitable agent for the dissociation of blood clots from CVS. The effects on cell settling time and time to first harvest with samples either treated routinely or by dissociation with ACR were compared.

Materials & Method
A study of 25 CVS samples treated with ACR was compared with a control group of 25 samples processed by conventional dissecting methods. Samples receiving ACR treatment were selected on the basis of quantity of villi and degree of blood clot present. (photo 1) Treated samples were bathed in 5mls of ACR solution (2 mg/ml in PBS).

Dissociation of blood clots from tissue was assessed by eye. To facilitate this process, the sample was gently agitated with a Pasteur pipette every 10 minutes until blood had dissociated. The majority of ACR treated samples required between 10 and 30 minutes treatment to fully dissolve blood clots. Washing the sample in PBS removed excess blood (photo 2) leaving the cleared sample to be examined microscopically for the presence of fetal material.

All samples were then treated using the standard laboratory protocol for establishing cultures (modification of Smidt-Jenson et al).

After 3 days incubation, cultures were assessed and thereafter on a daily basis, for signs of settling and suitability for harvest.

Results
There was 100% success rate for karyotype analysis in both the ACR treated and control groups. On average, ACR treated samples took 20 minutes to clear blood clots, compared to the control group which took between 30 and 60 minutes to clear with needle dissection. (Times are valid where comparable extent of blood clotting was present).

Data on settling time and time to harvest is shown below.

Conclusions
• In this current study, ACR pre-treatment of the samples replaced the need for needles to remove blood clots, thus eliminating the risk of needlestick injury to the operator.
• ACR was found to significantly decrease the time taken for cell settling (p=0.0008) and time to first harvest (p=0.0111). Cytogenetic preparations were therefore available faster than standard culture procedures, with the resulting potential to reduce reporting time and patient anxiety.
• ACR pre-treatment was a simple procedure which had the additional advantage of reducing sample processing time in the laboratory. It was noted that 3 out of 25 non ACR treated samples showed evidence of MCC. There was no evidence of MCC in ACR treated samples. (Although this sample size is too small to prove if ACR has any statistically significant effect on MCC, further studies are continuing).

References
1) Advisory Committee on Dangerous Pathogens (2001), Revised Advice on Laboratory Containment Measures for Work with Tissue Samples in Clinical Cytogenetics Laboratories (Supplement to ACOP guidance on protection against blood-borne infections in the workplace): HSE books.