In 1994, the POMBA Canada (MBC’s predecessor) Board of Directors launched its Zygosity Testing Program and promoted this service to its affiliates nationwide. The Zygosity Testing Program came about through the work of Dr. Geoffrey Machin, a pediatric pathologist at the University of Alberta, who was a POMBA Advisory Board Member at that time. Through his work as a pathologist, Dr. Machin saw an increasing number of multiple-birth fetuses that had succumbed to twin-to-twin transfusion syndrome.

Being a scientist, he began asking questions about multiple births. Why does the egg split? What causes it to split? What happens in twin-to-twin transfusion? How can medicine prevent this? ...and so on. Dr. Machin discovered many parents of multiples had inconclusive answers regarding their multiples’ zygosity; something he believed every family had the right to know. This program continues to exist today, under the commitment of Multiple Births Canada (formerly POMBA Canada) and Dr. Fiona Bamforth, University of Alberta Hospital.

It is Multiple Births Canada’s belief that every parent and every multiple has the right to know their zygosity. Through Multiple Births Canada’s National Zygosity Testing Program, parents and multiples will have the answers to their questions, “Are they fraternal or identical?”

Facts and Information on Multiples & Zygosity Testing

Multiple Births Canada encourages the use of correct terminology regarding zygosity.

Monozygotic twins—commonly called identical twins—are genetically alike but never completely identical in appearance or personality. Therefore the term “identical” should be discouraged. These twins come from one fertilized egg, which has split—once for twins, twice to produce monozygotic triplets, etc.

Dizygotic twins—commonly called fraternal twins—are born from two separate fertilized eggs. Fraternal means brotherly—dizygotic twins can be made up of a set of boys or a set of girls or one of each sex. Therefore the term “fraternal” should be discouraged.

Trizygotic multiples are a set of triplets born from three separate fertilized eggs and can be any combination of both sexes. Quadrazygotic - four separate fertilized eggs; quintrazygotic - five separate eggs, etc. Triplets and other higher-order multiples may also be a combination of both monozygotic & dizygotic children.

One-third of monozygotic twins and all dizygotic twins have two placentas, separate or fused. Two-thirds of monozygotic twins share one placenta (chorion). Almost all twins have two sacs (amnions) – with the exception of extremely rare twins who have one chorion and one amnion.

Parents can ask questions about zygosity determination when the woman is pregnant. Ultrasound can demonstrate two key criteria in helping parents determine zygosity: determining through visual checks whether the fetuses are female or male, and determining the thickness of the septum – the thicker the septum the more likely the twins are dizygotic.
How is zygosity testing done?

Zygosity is determined by examination of deoxyribonucleic acid, better known as DNA. DNA is the chromosomal material responsible for transmitting hereditary traits. For zygosity testing, the most convenient sample is a cheek swab sample collected from each twin/multiple by gently wiping the inside of the mouth with a soft brush supplied by the laboratory. This can be done by parents or by older children themselves and is quite painless. Blood samples can also be used but require a blood poke and are less convenient to send to the laboratory.

How does DNA zygosity testing work?

First, DNA has to be isolated from the cheek swab or blood samples. Up to 13 small fragments of DNA (short tandem repeats or STRs) that are very different between different individuals are examined by a technique called PCR (Polymerase Chain Reaction). Each person has a unique pattern of fragments unless they are monozygotic multiples. Dizygotic twin pairs have different patterns.

Why should we have our multiples DNA tested?

DNA testing is 99.99% accurate. If the zygosity is in question this is the most accurate method to determine whether the multiple sets are monozygotic or dizygotic. Parents report that conclusive answers have helped them with social issues, educational issues and even medical issues concerning multiple-birth children.

Who would be eligible for Zygosity testing?

The test is available to any set of multiples whose zygosity is in question. Even today, many mothers are told their multiples are dizygotic based solely on the fact that two placenta were present at birth. This, simply, is not true. Many parents report they believe their multiples are monozygotic despite what the doctor told them. The most common scenario for DNA testing is the family who has been told their multiples are dizygotic (based on the presence of two placentas) and yet, the multiples are similar looking and many family members and friends have difficulty telling the multiples apart. Many people still believe that monozygotic multiples must look exactly alike. This is also not true. Many factors influence the growth of babies. Another common story is when the zygosity is called “inconclusive”.

What do I need to do to get the DNA test done?

Cheek swab samples
Contact the laboratory genetic counsellor by phone. The collection brushes and instructions will be sent by mail. Also included are a requisition for testing and a consent form. Once collected, the samples should be mailed by Expresspost to the laboratory within three days of collection. Because cheek swab samples are not collected in a laboratory setting, results cannot be used for medical or legal purposes.

Blood samples
Families must obtain, from a physician, a general requisition requesting zygosity testing. One 2 mL EDTA lavender-topped blood sample must be obtained from each multiple. The blood should be sent by courier to arrive at the laboratory within three
days of the collection. It is best to have the blood drawn on a Monday / Tuesday so it doesn’t get held up over a weekend. Please note that there are Federal regulations for transport of blood samples and you should ask your local laboratory for information before the samples are collected.

Results are sent both to the doctor who requested the test and to the parents or multiples. (Please be sure to include the name and address of the family physician).

**My doctor says it isn’t necessary, and is far too expensive.**
Multiple Births Canada believes every multiple-birth parent and multiple has the right to know his or her zygosity. Families have the right to know and the right to ask for the test. Zygosity testing is not covered by provincial healthcare programs and is normally extremely expensive (reports range from $500-$800 for the test.)

Multiple Births Canada’s Zygosity Testing Program is available for all multiple-birth parents and all multiple-birth children. The cost of the test for twins is $350 (subject to change). Please make your cheque payable to University of Alberta. For blood samples, there may be an additional charge for the blood collection.

Please send samples, cheque and completed information to:

Dr. Stacey Hume,
8-26 Medical Sciences Building,
University of Alberta,
Edmonton, Alberta, T6G 2H7

Please allow 6 - 8 weeks for results to be mailed.

If you require more information, please contact the laboratory genetic counsellor at (780) 407-1015.

**Recommended Sources of Information:**
Multiple Births Canada
www.multiplebirthscanada.org

Documents and Articles:
Multiple Births Canada Fact Sheets on various topics related to multiple pregnancy, births and parenting multiples.
multiplebirthscanada.org/index.php/education/publications/fact-sheets:

- Incidence of Multiple Births
- Biology of Multiples
- Multiple Birth Facts and Figures
- Multiple Birth Terminology

**Multiple Births Canada**

**Reviewers:**
Susan Christian, MSc, CGC, Genetic Counsellor
Pamela Blumenschein, MSc, CGC, Genetic Counsellor
Margaret Lilley, MSc, CGC, Genetic Counsellor
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