Welcome to Mayo Medical Laboratories Profiles in Genetics. These presentations provide short discussion of current genetics topics and may be helpful to you in your practice. This presentation discusses the various options for prenatal diagnostic testing.
Our speaker for this program is McKinsey Goodenberger, MS CGC, a Genetic Counselor in the Cytogenetics Laboratory at Mayo Clinic, Rochester, Minnesota. In this presentation McKinsey, thank you for presenting with us today.
Thank you for the introduction. I have nothing to disclose.
Disclosures

• Nothing to disclose

Script: Thank you for the introduction. We have nothing to disclose.
To get us started today, I thought I’d quickly review the indications where prenatal genetic diagnostic testing might be indicated. These indications for genetic testing might be known up front in the pregnancy or might arise during the pregnancy. One of the biggest reasons for prenatal diagnostic testing is aneuploidy. Aneuploidy is having an extra or a missing chromosome, such as Down syndrome. All pregnancies have a baseline risk for aneuploidy, and this does increase with maternal age. So, many families will want to do prenatal diagnostic testing for the risk for aneuploidy as soon as they know they’re pregnant. Other indications for prenatal diagnostic testing might be carrier screening. All couples are offered carrier screening based on their ethnicity. In the last few years, there’s been many panels that offer genetic testing for carrier status for recessive conditions, for many conditions; and if both mom and dad are found to be mutation carriers of the same condition, then the pregnancy will have a 25% chance of actually being affected, which could lead to an offer for prenatal genetic diagnostic testing. And, rarely, some families will have a family history of a genetic mutation or a known genetic condition. All of these would be known up front in the pregnancy and can be offered testing throughout the pregnancy. And then, unfortunately, some pregnancies, the need or offer for prenatal diagnostic testing will arise during that pregnancy. A common indication is when the prenatal screening for aneuploidy comes back abnormal. This would include maternal serum screening or cell-free DNA screening. If this comes back indicating a higher risk for aneuploidy, then prenatal diagnostic testing would be the next step to either confirm or rule out the finding. And on ultrasound, we might see birth defects, such as heart defect, extra fluid, other soft
markers that might indicate another increased risk for aneuploidy, and all of these things might lead to the offer for prenatal diagnostic testing.
Prenatal diagnostic testing can be done largely on 2 different specimen types, either chorionic villi or amniotic fluid. Chorionic villi is part of the placenta and as the placenta and the baby come from the same cell, in the vast majority of cases, both the chorionic villi and the baby will have the same genetic content, which is why we’re able to test chorionic villi. We can collect this villi between the ninth and fourteenth weeks of pregnancy. And then after 15 weeks, we can collect amniotic fluid. Within that amniotic fluid, we will find shed skin cells from the baby. It’s natural for the baby to shed skin, and it’s actually that skin that we then test for genetic testing.

<table>
<thead>
<tr>
<th>Prenatal Specimen Types for Diagnostic Testing</th>
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<tr>
<td><strong>Chorionic villi</strong></td>
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<td>• Collected between 9th and 14th week of pregnancy</td>
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<td><img src="image" alt="Image of chorionic villi collection" /></td>
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<td><strong>Amniotic Fluid</strong></td>
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<td>• Collected after 15 weeks</td>
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Once the specimens are received in the laboratory, there’s 3 main ways we might prepare that specimen. One very limited way is an interphase pellet. We can only do FISH from this, but the good part of an interphase pellet is it only takes about 1 hour to prepare, and then we can start the actual testing process. The vast majority of specimens that come into our laboratory, though, are prepared for culture. And the reason for that is you can really do almost any type of genetic testing from a culture, culture meaning we get the cells growing, we’re going to have metaphases, etc. And from that culture, we can do FISH, chromosome analysis, microarray, or molecular studies. The downside of culture is it does take quite a bit of time. So in order to get these cells growing to the state that we need and the volume that we need, usually these amniotic fluids and chorionic villi take about 7 to 10 days in culture, and that’s even before the testing can get started. And in the last year or 2, we’ve started doing direct DNA extraction from these prenatal specimens. This can be used for microarray and, in some cases, molecular studies. This is much faster than culture. We do have a DNA sample in about 36 hours after a prenatal specimen arrives. I should mention that it’s slower than direct extraction from a blood specimen or postnatal specimen as a prenatal specimen does require an overnight digestion and hand extraction, but 36 hours compared to 7 to 10 days is much faster. Unfortunately, though, the amount of DNA is much smaller than what you would get from the cultures eventually.

### Specimen Preparation

- **Interphase pellet**
  - FISH
  - ~1 hour

- **Culture**
  - FISH, chromosomes, microarray, molecular studies
  - 7-10 days

- **Direct DNA extraction**
  - Microarray, molecular studies
  - ~36 hours

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So once we have our specimens prepped, then to go through the different types of genetic testing we might be able to perform on them for prenatal diagnostic. The only test that we offer on the interphase pellet is our aneuploidy detection FISH, a test called PADF. And this tests for the most common aneuploidies in human pregnancy, namely trisomy 13, 18, 21, and then the sex chromosomes X and Y. Since we are doing this on an interphase pellet, though, as long as we get the specimen in from Sunday to Thursday, the results will be available Monday through Friday. So it’s a 24-hour test, which is the main benefit of this study in that we get an answer in 24 hours, but there’s some major limitations. So, examples on your screen here is a case with what we would call positive for trisomy 21. If you look at the image on the left, there are 3 red signals, that’s 3 probes, and that probe is attached to the long arm of chromosome 21. This result, though, is not diagnostic. We would say this is concerning, and it’s most likely trisomy 21. But as you can see from these FISH images, we are not getting any structural information on these chromosomes. All we know is that this specific location on 21, there’s 3 copies. Now the most likely explanation is this is Down syndrome and there’s 3 copies of 21 there, but we can’t tell if they’re 3 separate copies of chromosome 21 or if the extra copy of 21 is attached to a different chromosome, and very rarely, it might just be a duplication of the 1 region on 21 that the probe is attaching to, and it’s not Down syndrome at all but a different genetic abnormality. None of this can be told from this interphase pellet and this FISH study. So, all FISH results that are done on an interphase pellet will need confirmation studies either by chromosomes or microarray.
We very rarely do this, but we do have FISH for microdeletion syndromes in the laboratory. This might be appropriate if an ultrasound finding is very specific or there’s a known family history of something such as William syndrome. FISH for microdeletion syndromes do need to be done on metaphase cells, and that’s what your image is there. When you’re doing FISH on metaphase, you can actually see the actual chromosomes, so you do get some structural information there. When we do a FISH for microdeletion syndrome, it takes us about 2 to 3 days for the probe hybridization and then analysis, but that is going to require a culture so that we can get those metaphase cells. So with that culture time, the total turnaround time is about 10 to 14 days.
Up until a couple of years ago, the most common genetic test done for prenatal diagnosis was the standard chromosome analysis or a karyotype. We have 2 test codes for this—CHRCV when it’s done on chorionic villi, and CHRAF when it’s done on amniotic fluid. And the chromosome analysis is going to give us the fetal karyotype, and that’s what you’re seeing on the right there; that’s the karyogram after a technologist has captured the image of the chromosomes under the microscope. The image on the right is a normal 46,XX, indicating a female sex, and if it was male, it would be 46,XY. Indications for a chromosome analysis is when you’re trying to rule-out aneuploidy or very large chromosome rearrangements, and occasionally, chromosome analysis is still done for abnormal ultrasound per a patient or physician’s preference.
### Chromosome Analysis

- **Requires cultures/metaphase cells**
  - 2-3 days for staining and analysis
    - TAT (with culture): 10-14 days

- **Benefits**
  - Structural information
  - Low risk for uncertain or incidental findings

- **Limitations**
  - Culture required
    - Potential for cultural artifacts
    - TAT
  - Low resolution

The thing about chromosome analysis, it does require cultures, those metaphase cells. Once the specimen is done growing, the actual staining and analysis of chromosomes only take about 2 to 3 days, but with the culture, the total turnaround time is going to be closer to 10 to 14 days. The main benefit of a chromosome analysis is that you are going to get that structural information. So you’re not just going to see—are there 3 copies of chromosome 21? You’ll be able to see—are they 3 separate copies of 21, or is this an unbalanced rearrangement? You get that structural information. The other benefit of chromosome analysis is there’s a very low risk for uncertain or incidental findings, less than a 1% chance. Many people who are being tested for things such as aneuploidy, given maternal serum screening, if that’s abnormal, they aren’t necessarily looking for other things in their genetic diagnosis that we can’t quite explain. And the chromosome analysis is really going to have a low risk of finding those incidental or uncertain findings. Limitations of chromosome studies though, it is taking that culture, which is going to be a long turnaround time-10 to 14 days, and whenever you’re culturing cells, particularly in the prenatal setting, there is a potential for cultural artifacts. And then the biggest limitation for chromosomes is it does have a low resolution. So in order for us to actually see an unbalance in chromosomes or a deletion duplication, it has to be bigger than 5 megabases and possibly even bigger than that, given the banding resolution on a prenatal specimen. So for things like Down syndrome, where there’s an entire extra chromosome, we can easily see that on chromosome studies, but there’s a lot of deletions and duplications that are clinically significant that a chromosome study just doesn’t have the resolution to detect.
Prenatal Microarray

- CMAP / Chromosomal Microarray, Prenatal, Amniotic Fluid/Chorionic Villus Sampling
  - Chorionic villi or amniotic fluid
- Diagnosis of small deletions and duplications across the genome associated with birth defects, intellectual disability
  - Also detects excessive homozygosity
- Utilizes >1.9 million copy number probes and ~750,000 SNPs probes

Which brings me to the prenatal microarray, our test code is CMAP, and that can be performed on chorionic villi or amniotic fluid, and this test will pick up very small deletions and duplications across the genome down to about 100 Kb, so it has a much higher resolution than the chromosome study. It will also pick up excess homozygosity, also known as absence of heterozygosity, and this is when both copies of the chromosome in a region are more identical than they are different. Our microarray utilizes greater than 1.9 million copy number probes and approximately 750,000 SNP probes, and this large number of probes is very important. It gives us a very in-depth and broad coverage across the genome, and this helps us with our microarray to appreciate very subtle or even complex abnormalities, even though we’re not actually seeing the structure of the chromosomes.
Just for reference, this would be what our technologists are looking at. This is our normal profile on the prenatal microarray, and I really put this in here to highlight that the microarray is a computerized analysis, unlike FISH and chromosomes, which is done by microscope.
What we actually report out on our prenatal microarray, we report out anything that’s pathogenic, so anything with clinical significance that’s known, we will report it. That includes adult-onset conditions. So even if it’s not related to why the prenatal diagnostic testing was originally ordered, if we find something on this test that we know will cause a clinical consequence or phenotype, it will be reported. We also report out regions of absence of heterozygosity if they’re fairly large and on chromosomes that we know have imprinting or UPD-associated effects. We can also see absence of heterozygosity throughout the whole genome, and if it’s greater than 10%, we will also report it. And this would be a strong indication for a somewhat high level of consanguinity in the family. So it’s another thing that can be reported on the prenatal microarray. And with the microarray, when you have that higher resolution, there’s also a higher chance of finding deletions, duplications, that we’re not sure if they actually have clinical significance. So uncertain deletions that are greater than 1 Mb or uncertain duplications that are greater than 2 Mb, if they contain a gene, will get reported.
How we follow up on these abnormalities that we find, as long as they’re big enough, we will do FISH studies to confirm. And in cases of uncertain deletions and duplications, we do offer a way to send us in parent specimens, and we will do testing on mom and dad at no charge. What the idea is, if mom and dad are carrying this duplication that we’re seeing in the fetus and they don’t have any clinical features—normal intelligence, no birth defects—then it would give one line of evidence that possibly this duplication is not going to cause a problem in the fetus. Now that’s not confirmatory, but it is one line of evidence. Now on the other hand, if mom and dad are not carrying the duplication and it’s a de novo finding in the child, then it might have more significance in that it could determine a clinical outcome although, again, not confirmatory but it’s another line of evidence. We actually will take parental specimens up front if they want to be sent in with the actual prenatal specimen just in case an uncertain finding is found. This allows us to get that final report with parental information done as soon as possible for the clinician, just because many of these prenatal specimens have a timeline for the pregnancy. So we try and do that as fast as possible. When we do pick up the standard aneuploidies on microarray, we confirm those by FISH, except for trisomy 13 and 21. We will wait and do a limited chromosome study on metaphases since trisomy 13 and 21, those chromosomes are acrocentric and can be involved with robertsonian translocations, we will look at them structurally so that we can rule out an unbalanced rearrangement, which might influence recurrence risks.
We prefer to perform the microarray on directly extracted DNA. This data is much cleaner, and it removes the concern for cultural artifact. It also has a much faster turnaround time. If we are successful with directly extracting DNA, we can have a microarray result in 5 to 7 days, so much faster than the standard karyotype.

Unfortunately, though, given the small amount of DNA that we will find, particularly in amniotic fluid, we occasionally will not get enough DNA from the direct extraction in order to be successful with our microarray, or the quality control will fail and we need to reset the case. So, unfortunately, for a certain number of prenatal microarray cases that come into the laboratory, we will need to reflex to a culture. Also, these prenatal tests, if the physician has ordered many different tests we might not receive the volume needed that we would require for direct extraction and performing cultures needed for other studies. So, some microarrays will need to be done from culture, and if we do need to culture first, then that’s going to increase the turnaround time to 10 to 14 days, more along the lines of the standard karyotype. Limitations of prenatal microarray is that we’re not going to pick up balanced rearrangements. It only finds deletions, duplications, essentially unbalanced rearrangements, and we’re not going to pick up all cases of mosaicism. And particularly in comparison to a chromosome study, the prenatal microarray has a much higher incidence of uncertain or incidental findings, and because of that, ACOG actually has guidelines on how a patient should be counseled up front before having a prenatal microarray performed.

Prenatal Microarray

- Preferable that microarray is performed on directly extracted DNA
- TAT of 5 to 7 days
  - Direct extraction failure/resets
  - If from culture, TAT will be greatly increased (10-14 days)
- Limitations
  - Balanced rearrangements, all cases of mosaicism
  - Uncertain/incidental findings
    - ACOG guidelines on patient counseling

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And just to briefly touch a bit more on prenatal microarray vs. chromosome analysis, as this has been a recent clinical change, in 2013, ACOG recommended prenatal microarray rather than chromosome studies when you’re evaluating a pregnancy with a structural abnormality on ultrasound. The reason for that was largely due to the higher resolution. A microarray is going to detect aneuploidies and unbalanced rearrangements as chromosome analysis could, but it will also pick up an additional 6% of clinically significant deletions and duplications in pregnancies when there is an abnormal ultrasound. And because of that higher resolution, ACOG is therefore recommending microarray rather than the chromosomes when you are seeing something on ultrasound. On the other hand, if you’re doing prenatal diagnostic testing and the ultrasound does not have a structural abnormality, then ACOG says either chromosomes or microarray is appropriate based on patient preference. Another benefit, though, of the microarray again, is that you do not need cultured cells for most cases, which makes it faster, and you’re not going to have those culture complications, such as culture artifact or the culture failure.
And then just to briefly wrap up, in some pregnancies, although it’s not as common, there will be a need for molecular studies. This is when there’s ultrasound findings that are suggestive of a particular genetic condition where the mutations would be on the molecular level or in the sequence structure, so again, way above the resolution of a microarray in that it’s a single-base pair change, etc. Or maybe there’s a family history of a known genetic condition, and you need to test the pregnancy for that genetic condition. Currently here at Mayo Clinic, our molecular studies need to be done on cultured cells in order to get enough DNA. So 2 additional tests will need to be ordered—the CULAF if it’s an amniotic fluid, or a CULFB if it’s a chorionic villi, in order to grow out the cultures needed. And then the DNA is extracted from those cultured cell pellets, and we do recommend maternal cell contamination studies for our molecular tests.
## Molecular Studies

- **TAT**
  - 7-10 days for the culture, 2-4 days for extraction from cell pellet, 2-3 weeks molecular analysis

- **Restrictions:**
  - Studies requiring methylation analysis cannot be performed on chorionic villi
  - Not available for adult onset conditions
  - Familial mutation required

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Turnaround time for molecular studies is fairly significant because there’s many steps. You need that 7 to 10 days in culture, and then extraction is going to be 2 to 4 days from that cell pellet, and then that’s when the actual molecular analysis begins, which is a standard 2- to 3-week turnaround time, which gives a total turnaround time closer to 4 to 5 weeks in order to get a molecular test result. There are some restrictions on molecular studies as well, studies that require methylation analysis, such as our Beckwith-Wiedemann testing and some others, we cannot do those on chorionic villi reliably, and they are only available for amniotic fluid. We don’t routinely offer genetic testing in the prenatal setting for adult-onset conditions in the vast majority of cases. When the reason for the molecular studies is because there’s a family history of a genetic condition, the familial mutation must be known in order for us to proceed with the testing. So those would be the main restrictions of our molecular studies here at Mayo Clinic.
This concludes my talk. I appreciate your attention.